U. S. ARMY. 26th HOSPITAL CENTER

CHEMICAL DETERMINATION OF NUTRITIONAL STATE OF FULL DUTY TROOPS, MANILA AREA, JULY AND AUGUST 1946

HEADQUARTERS
U.S. Army, 26th Hospital Center

APO 75
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SUBJECT: Chemical Determination of Nutritional State of Full Duty Troops, Manila Area, July and August 1945.

TO : Commanding Officer, 26th Hospital Center, APO 75.

1. The attached report presents an intensive study on the nutritional status of one hundred and eleven (111) full duty troops selected from eleven (11) units within the command. The principal data have been secured through the application of newer biochemical methods for the estimation of vitamins in body fluids. These data have been supplemented by clinical and other observations that contribute a fuller understanding of nutritional condition.

2. The results of this integrated study are presented in detail in the several inclosures which follow. Reference is made to Inclosure 1, "Summary and Conclusions" for a review of the more salient findings.

3. The report is of immediate interest as a nutritional study on representative troops in this area. The study has more fundamental scientific value as it deals with the general concept of nutrition in relation to health and demonstrates the type of information that will be secured as new methods are brought to bear on nutritional problems.

4. The chemical data show that some men in the experimental group are in a state of partial depletion with respect to one or more nutritional factors in degrees believed to be "severe" to "borderline". This in no way implies criticism of the current ration or army feeding practice. Nutritional condition is the resultant of many other complex factors entirely unrelated to food supply.

5. The study further shows that the group classified as being depleted in some degree with respect to Vitamin B_1 (thiamine) and Vitamin B_2 (riboflavin) exhibit certain undesirable physical characteristics indicative of lowered physical health. There were, however, no clinical symptoms diagnostic of specific nutritional deficiency in any of the subjects.

6. This type of observation, if it can be confirmed in more extensive studies in the future, will result in a clearer understanding of the way in which nutrition is related to physical health and sense of well-being.

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Inclosure 1

Summary and Conclusions

1. A group of one hundred and eleven (111) full duty troops, selected at random in the Manila Area, have been studied with reference to their nutritional status. New chemical methods have been used to secure the data and further scope has been added to the study with pertinent clinical and other observations.

2. Chemical methods consisted of the following:

   a. Fasting urinary excretion of sodium chloride, ascorbic acid, (Vitamin C), thiamine (Vitamin B_1), and riboflavin (Vitamin B_2).

   b. Urinary excretion of thiamine and riboflavin after oral administration of test doses of Vitamin B_1 and B_2.

   c. Whole blood and plasma concentrations of ascorbic acid.

   d. Hemoglobin and plasma protein concentration in blood.

The chemical methods are reviewed in Inclosure 4 with emphasis on the ways in which the original methods have been simplified and adapted for obtaining significant data with the limited amount of special equipment available in the present work.

3. The results presented in Inclosures 5, 6 and 7 may be summarized as follows:

   a. Considerable variability exists in nutritional state of troops exposed to similar environment, living conditions and basic ration allowance.

   b. The ration provided for troops in this area at the time of study appeared adequate according to routine estimates through possibly richer in calories than average soldier requires under tropical conditions. The present experiment does not test the effect of current ration on

nutritional state, for dietary is only one of the many factors involved; such as, past history of infection and therapy, individual dietary habits, differences in metabolism, effect of tropical environment, psychological makeup and exposure to unsatisfactory dietary in the past in civilian or army life.

c. Chemical tests interpreted in accordance with the best available methods suggest that:

(1) Twenty-five (25) per cent of the subjects were in a state of partial depletion with respect to sodium chloride.

(2) Plasma protein concentrations were normal and no serious deficiency of hemoglobin existed although sixteen (16) per cent of the subjects were slightly below accepted normal standards.

(3) Twenty-five (25) per cent of the subjects had blood concentrations of Vitamin C below 0.4 milligrams per 100 cc, an indication of reduced body stores of the vitamin. No blood concentrations suggestive of the "prescurvy state" were observed.

(4) Nine (9) per cent of the subjects appeared to be rather seriously depleted of Vitamin B_1 (thiamine) and others showed evidence of approaching this state of desaturation.

(5) Six (6) per cent of the subjects were classed as "seriously" depleted with respect to riboflavin.

(6) Average performance of all subjects in the chemical tests is consistently lower than the average performance of normal troops in the States. This observation is suggestive of a lowered plane of nutrition. Whether such a lowered plane of nutrition is detrimental to the troops cannot be decided on the basis of present knowledge. Exposure to tropical environment with lowering of me-

tabolism and food intake may be the principal cause of lower performance in the tests.

d. State of nutrition with respect to B-vitamins (thiamine and riboflavin) in these experiments showed no relation to:

(1) Height, weight, or body-form.

(2) Age

(3) Months of overseas service.

(4) Plasma protein concentration.

(5) Nutritional state with respect to Vitamin C or sodium Chloride.

e. Blood hemoglobin tended to be lower in subjects giving evidence of depleted stores of either Vitamin B_1 or Vitamin B_2.

f. Low body stores of thiamine are usually accompanied by relative desaturation in riboflavin.

g. Subjects evaluated their morale, condition of appetite and state of health (medical history for previous six (6) months) in confidential questionaires. When subjects giving evidence of lowered state of B-vitamin nutrition were compared with subjects in excellent nutritional state, it was found that the less well nourished subjects recorded poorer morale, poorer appetite and greater frequency of acute infections. The interpretation of these results is not entirely clear but suggested (but not proven) that the more poorly nourished subjects were characterized by lowered sense of well-being.

H. Clinical differences were evident when twenty-seven (27) subjects making low scores in B-vitamin tests (presumably more or less depleted of B Vitamins) were compared with 16 subjects making high scores in the B-vitamin tests. Thus, in the low score group there was a greater incidence of:

(1) Hospitalizations and sick calls in past 6 months.

(2) Acute respiratory infections in the past 6 months.

(4 times as many attacks per man)

(3) Acute diarrheas in the past 6 months.

(10 times as many attacks per man)

(4) Anomalies in knee and ankle jerk reflexes.

(5) Complaints of headache, insomnia, sycope on arising.

(6) Paresthesias.

(7) Palpable livers of significant degree.

(8) A peculiar lesion not believed due to ringworm in the sulcus above the anus.

Common signs of specific avitaminoses were not found in any of the subjects nor was corneal vascularity (suggestive of ariboflavinosis) observed. Skin disorders were not significantly more frequent in the low score subjects.

1. Interpretation of these results indicates that, in the present experiment, low score subjects (partially desaturated with respect to the vitamin B complex) were characterized by a greater incidence of non-specific signs of lowered health, resistance and sense of well-being. It is not clear what part hypochondria and other psychological states may play in the present findings. Likewise it is not clear whether lowered nutritional state acted as cause or effect in its relation to incidence of infectious disease and other signs of pathology. The results do indicate many lines of future research which could be followed profitably using the chemical methods to evaluate the nutritional status. Foremost among these problems are:

(1) Relation of mildly lowered nutritional state to acute intestinal infection.

(2) Psychological factors involved in nutritional status.

Inclosure 2

Organization of the Study

1. The present study has been carried out in the 26th Hospital Center under command of Paul Ireland, Col, MC, for the purpose of securing fundamental information on the nutritional condition of troops. The experimental work has been organized and all of the chemical analyses and interpretations have been made by Eliot F. Beach, Captain, Sn C, Nutrition Officer, 26th Hospital Center and Oscar N. Miller, 1st Lt, Sn C, Nutrition Officer, 248th General Hospital. Collection of urine and blood samples and data in questionaires was made by Fred Draeseke, 2nd Lt, Sn C.

2. Clinical examinations of subjects were generously contributed by:

    a. Bertram Nelson, Major, MC, 13th General Hospital.

    b. Richard Morris, Captain, MC, 13th General Hospital.

    c. Frank Green, Major, MC, 60th General Hospital

    who examined subjects with the slit lamp for evidence of eye pathology related to avitaminotic states.

    Interpretations of the clinical data have been made with the advice of these officers.

3. Cooperation of Unit organizations participating in the study was secured with the assistance of Josep Weybrew, 1st Lt, Sn C, Nutrition Officer, Base X. Lt Weybrew also contributed nutritional evaluation of rations drawn by unit organizations during the period of study.

4. Laboratory facilities, standard apparatus and reagents were placed at the disposal of the study by the Commanding Officer, 363d Medical Laboratory, University of Santo Tomas and James C. Harris, Captain, Sn C, in charge of

the Biochemical Section.

5. Preliminary standardization of the chemical methods used in this work was carried out by one of us several months ago in Brisbane, Australia, with the permission and encouragement of Carl Mitchell, Col, MC, Surgeon, Australian Base Section USASOS. At that time special reagents and chemicals required for the present study were assembled.

6. Unit organizations who participated by contributing subjects were:

4037th Quartermaster Co.

253rd Ordnance (M) A.A. Co.

4168th Quartermaster Depot Co.

151st, 242nd, 247th, 248th and 249th Port Cos.

911th A.A.A., Air Warning Bn, Hq Btry

874th Engineering Aviation Bn

738th Military Police Co.

276th Signal Construction Co.

363rd Medical Laboratory

120th General Hospital

Base X, Headquarters Co.

7. Approval of the research as a part of the Nutrition Program and encouragement in its execution were given by:

a. Seward Owen, Lt Col, Sn C, Nutrition Officer
Headquarters, USASOS

b. William Bergen, Major, Sn C, Nutrition Officer
Headquarters, AFWESPAC

c. Burr Ross, Captain, Sn C, Nutrition Officer
Headquarters, PHIBSEC

8. Without the support of many officers and organizations this integrated study would have been impossible.

Inclosure 3

Collection of Samples

Technical details in the chemical methods were such that only ten or twelve subjects could be studied at one time. Eleven units contributing subjects were studied successively to obtain all the observations. Collection of samples in each unit organization was made by the following routine:

1. Ten subjects were selected at random from the company roster without regard to rank or other visible characteristics.
2. On the afternoon prior to the first experimental day ten (10) subjects were brought together and given instruction on the general procedure that would be used. At this time all subjects who had been taking vitamin tablet supplement were eliminated from the study and other subjects chosen. Thus, all troops in the study had no dietary source of vitamins other than food for at least six (6) months prior to the experiment.
3. Supper was the last meal permitted until all fasting samples had been collected on the following morning.
4. On the morning of the first experimental day the subjects were awakened and assembled in the tent or building set aside in the company area for collection of samples. All subjects urinated at a specified time to secure complete emptying of the bladder in preparation for the fasting urine sample The urine excreted at this time was discarded.
5. During the 4-hour fasting period which followed, venous blood samples (10 to 15 cc) were taken from the subjects. Blood samples

were stored in citrated tubes. During this time, also, the subjects were employed in filling out protocols containing pertinent data, and confidential questionaires relating to morale, appetite and medical history for the six (6) months prior to experiment. No food was allowed during the fasting period. High temperature in the tropics makes securing of urine samples a special problem. It was found that if the subjects consumed about a pint of water during the fasting period the 4-hour urine samples would vary from one hundred to two hundred and fifty (100 - 250) cc, a convenient urine dilution for the chemical methods.

6. At the end of the 4-hour fasting period all subjects urinated into containers (large paper cups were found convenient) to complete bladder emptying. Each urine volume was measured in a graduated cylinder and the volume recorded. Exactly one hundred (100) cc of the urine were placed in a bottle containing three (3) cc of glacial acetic acid. Allowance was made in the subsequent chemical analyses for the dilution due to the acetic acid preservative.

7. Blood and urine samples were protected from direct exposure to sunlight and brought to the laboratory with the least possible delay. Analyses of the least stable factors (Urinary Vitamin C and B_2) were made at once.

8. The following day the same subjects were given oral load test doses of Vitamins B_1 and B_2. For this test the subjects were assembled at 0600 hours as on the previous day and bladders emptied. Each subject was then given 5 milligrams of thiamine hydrochloride and 5 milligrams of riboflavin by mouth. The test doses were immediately followed with breakfast which consisted of any items of usual food except eggs, bacon or other meat that might contribute any large amount of B-vitamins.

Bread, butter, jam, cereal, French toast, hot cakes, syrups, coffee and canned milk were among the items of food allowed. Breakfast was an essential feature of the load test inasmuch as oral doses of thiamine and riboflavin are but poorly absorbed unless taken with food. Test doses of thiamine and riboflavin were so large that the vitamin content of the breakfast could have contributed but little to the total vitamin intake during the test period.

9. Four hours after the test dose of vitamins the subjects urinated into containers to complete emptying the bladder. Samples were measured and stored as on the previous day and carried to the laboratory as soon as possible for analyses.

Inclosure 4

Chemical Methods

1. Urinary Sodium Chloride.

a. Sodium chloride estimations in fasting urine samples were made by the Volhard-Harvey method, p 769, Practical Physiological Chemistry, Hawk, P.B. and Bergeim, O., 11th Ed., Blackiston, Philadelphia. A few modifications were introduced to attain rapidity in performing the method.

b. Reagents needed for the method are:

(1) Acidified indicator.

To thirty (30) cc of distilled water add seventy (70) cc of thirty-three (33) per cent nitric acid (sp. gr. 1.2) and one hundred (100) grams of ferric ammonium sulfate.

(2) Standard silver nitrate solution.

Dissolve exactly 29.061 grams of silver nitrate (reagent grade) in one liter of distilled water. Each cubic centimeter of this solution is equivalent to 0.010 grams of sodium chloride or 0.006 grams of chlorine.

(3) Standard ammonium thiocyanate solution.

This solution is made so that one (1) cc is equivalent to one (1) cc of the standard silver nitrate solution described above. Make a concentrated solution of ammonium thiocyanate containing twenty-six (26) grams per liter. Determine the requisite dilution that will bring this solution to exact equivalence with the standard silver nitrate solution volume for volume. This may be done by titrating the silver nitrate solution with thiocyanate, using the acid indicator.

c. The procedure of the method is as follows:

Place five (5) to ten (10) cc of urine in an Erlenmeyer flask (125 cc), and dilute with twenty (20) cc of distilled water. Add two (2) cc of acid indicator and exactly ten (10) cc of standard silver nitrate solution to give an excess of silver ions above that required for complete precipitation of chloride. Back titrate the excess silver with standard ammonium thiocyanate solution until the first permanent red color forms, making the end point of the titration. Vigorous stirring is needed throughout the titration. It has been found simple and reasonably accurate to conduct the back titration by adding the standard thiocyanate solution by means of a five (5) cc graduated serological pipette instead of using the usual burette technique.

2. Protein Content of Plasma and Hemoglobin Concentration in Blood.

These estimates were made by the copper sulfate gravity method described in Quantitative Clinical Chemistry, p 940, Peters J. P. and Van Slyke D. D.; Vol II, Methods; Appendix June 1943 reprinting; Williams and Wilkins, Baltimore.

The technique consists of letting droplets of citrated plasma or whole blood fall into a graded series of solutions of copper sulfate of known specific gravity, and noting whether the droplets rise or fall in the solutions. Each drop becomes encased in a sack of copper proteinate and remains as a discrete drop without significant change in specific gravity for 20 seconds, during which, its rise or fall reveals its specific gravity relative to that of the solution. Size of droplets does not have to be regulated, temperature corrections are unnecessary and the method allows a high degree of accuracy.

Reference is made to the original source of this method for details of preparing the graded series of copper sulfate solutions. In the present

work solutions covering the specific gravity range of 1.023 through 1.030 in steps of 0.001 were used for plasma. The series for whole blood ranged from 1.052 to 1.070, differing stepwise in specific gravity by 0.002. Readings were made in both whole blood and plasma to the nearest 0.001. After 30 samples of blood had been tested, the graded series of copper sulfate tubes, containing one hundred (100) cc of solution, were renewed, thus avoiding the eventual error produced by repeated dilution with plasma or whole blood droplets.

The concentration of protein in the plasma and of hemoglobin in whole blood may be obtained from the specific gravity readings by reference to the nomogram line chart appearing in the original reference (page 952).

3. <u>Vitamin C in Urine</u>.

a. The reaction of ascorbic acid with 2,6-dichlorophenolindophenol forms the basis of Vitamin C estimation in urine. During this reaction the red dye in acid solution is quantitatively reduced to a colorless compound as the ascorbic acid is oxidized to dehydroascorbic acid. Ascorbic acid is a relatively unstable compound in urine. Acidification of the urine samples with glacial acetic acid assists in stabilizing Vitamin C so that the time lag of about an hour between collection of urine and its analysis for Vitamin C does not result in a severe loss of the vitamin. Small amounts of oxalic or metaphosphoric acids as preservatives in the urine would insure better protection of the ascorbic acid but they interfere seriously with other vitamin methods applied to the same urine samples. Therefore, these chemicals have not been used in the present work.

b. The only special reagent required for the Vitamin C estimation is a standard solution of 2,6-dichlorophenolindophenol. Standardization of this dye solution is based on the reaction of 2,6-dichlorophenolindophenol with potassium iodide, in acid solution, to liberate free iodine

quantitatively. The free iodine so liberated is titrated with standard sodium thiosulfate. The sodium thiosulfate solution is best standardized with potassium dichromate solution. Thus, potassium dichromate ultimately serves as the primary standard for the standardization of the dye. Because of the multiple steps involved in the standardization of 2,6-dichlorophenolindophenol solutions, the whole procedure is given below:

(1) Prepare 0.1 normal solutions of potassium dichromate and sodium thiosulfate using the potassium dichromate as primary standard. For the details of this procedure reference is made to pages 543 and 544, Handbook of Chemistry and Physics; Hodgman, C. D. and Lange, N. A., 16th Edition (1931), Chemical Rubber Publishing Col, Cleveland.

(2) Prepare a stock solution of 2,6-dichlorophenolindophenol by dissolving two hundred and fifty (250) milligrams of the powdered dye in one hundred and twenty-five (125) cc of a phosphate buffer of pH 7.2. (The phosphate buffer is made by dissolving 2.72 grams of potassium dihydrogen phosphate, $KH_2\ PO_4$, and 8.31 grams of crystalline disodium hydrogen phosphate, $Na_2\ H\ PO_4.2H_2$, in one (1) liter of water). The dye is not readily soluble at room temperature, but solution can be affected by extracting the powdered dye with successive small portions of the hot buffer and decanting these through a filter. After cooling the buffered dye solution to room temperature, filter again and store in the refrigerator. Stock indophenol dye solution of this strength is stable for two weeks if kept in a cool dark place. The dye solution, prepared as above, is equivalent to about 0.7 to 0.8 milligrams of Vitamin C per cc but its exact strength is determined through the standardization procedure which follows.

(3) Standardization of stock 2,6-dichlorophenolindophenol solution.

Place exactly two (2) cc of the stock dye solution in a fifty (50) cc Erlenmeyer flask and dilute with distilled water to about twenty (20) cc. Add 0.5 grams of pure potassium iodide (need not be exactly quantitative) and one (1) cc of dilute sulfuric acid (one (1) part concentrated $H_2 SO_4$ to three (3) parts of water). Shake the flask vigorously to facilitate the liberation of iodine. After two or three minutes, determine the quantity of liberated iodine by titration with 0.01 normal sodium thiosulfate solution (obtained by proper dilution of 0.1 normal sodium thiosulfate solution described above) using two (2) cc of one (1) per cent soluble starch solution as indicator. The titration, which amounts to between one (1) and two (2) cc, is done with a microburette. One (1) cc of 0.01 normal sodium thiosulfate is equivalent to 0.88 milligrams of Vitamin C (ascorbic acid). The exact Vitamin C equivalence of the stock indophenol is estimated from the titration value.

c. Titration of Vitamin C in urine with standard 2,6-dichlorophenolindophenol.

Titration is carried out with a solution of 2,6-dichlorophenolindophenol diluted to exactly one-tenth the strength of the standardized stock dye solution.described above.

Ten (10) cc of acidified urine are placed in a titration flask by means of a volumetric pipette (very dilute urines may require a sample twice this size). Add dilute standard 2,6-dichlorophenolindophenol by means of a microburette until the first faint pink color remains in the urine for fifteen (15) seconds. Judgement of the end point is facilitated by holding the flask against a well lighted white background and using

a second ten (10) cc urine sample as a color comparison. The fading end point in titration is caused by reducing substances, weaker than ascorbic acid (such as glutathione), which reduce with 2,6-dichlorophenolindophenol slowly after all of the ascorbic acid has reacted.

A second titration is performed in the same manner; only this time standard indophenol dye is added rapidly in a volume equal to that required in the first titration minus 0.2 cc. The end point is then carefully determined in this second titration and the volume of standard dilute indophenol dye is used as a basis for calculating urinary Vitamin C.

4. Vitamin C in Whole Blood and Plasma.

a. Estimations of Vitamin C in plasma and whole blood were made by the method of Roe and Kuether, Journal of Biological Chemistry 147, 399 (1943). Modifications have been necessary in the present work because of lack of equipment in forward areas. Speed and essential accuracy have not been sacrificed. The method is based upon a red color formed when strong sulphuric acid solution is added to the 2:4-dinitrophenylhydrazone of dehydroascorbic acid.

b. Reagents needed for this determination are:

(1) 2:4-dinitrophenylhydrozine solution.

Dissolve two (2) grams of 2:4-dinitrophenylhydrozine in nine (9) normal sulfuric acid (three (3) parts water and one (1) part concentrated sulfuric acid) and filter.

(2) Activated charcoal.

(3) Trichloracetic acid solution.

Dissolve six (6) grams of trichloracetic acid in water and dilute to one hundred (100) cc.

(4) Eighty-five (85) per cent sulfuric acid.

To one hundred (100) cc of water and nine hundred (900) cc concentrated sulfuric acid.

(5) Thiourea.

Dissolve ten (10) grams of thiourea in one hundred (100) cc of fifty (50) per cent aqueous alcohol.

c. Protein-free filtrate.

Place nine (9) cc of six (6) per cent trichloracetic acid in a fifty (50) cc flask and, with continuous stirring, add, dropwise, three (3) cc of whole blood or plasma to be analyzed. Let the mixture stand five (5) minutes and add one (1) gram of activated charcoal. Mix thoroughly and filter after letting the mixture stand three or four minutes. Treatment of the solution with charcoal converts all of the ascorbic acid to dehydroascorbic acid by oxidation with nascent adsorbed oxygen, which is liberated at the charcoal surface in contact with acid solution. The oxidation is rapid and quantitative.

d. Development of color reaction in protein-free filtrate.

Place three (3) cc of the charcoal-treated, protein-free filtrate in a small test tube of ten (10) cc cápacity and add one (1) drop of thiourea solution. Add 0.75 cc of 2,4-dinitrophenylhydrozine solution and mix the contents thoroughly. Place in a water bath maintained at 37.5 degrees for three (3) hours. Then cool in an ice water bath and add 3.75 cc of eighty-five (85) per cent sulfuric acid in small portions with continuous cooling. Rise in temperature during the addition of sulfuric acid must be avoided. Too much heat results in the charring of blood sugar and development of other interfering colors which may lead to difficulty in comparison with standard tubes.

After the addition of sulfuric acid, thirty (30) minutes is allowed for full development of the red color of the 2,4-dinitrophenylhydrazone before comparison with Vitamin C color standards described below.

e. Preparation of color standards.

In their original method, Roe and Kuether recommend the use of a photelometer for measurement of color intensity of the unknown solutions, readings of Vitamin C concentration being taken directly after the initial calibration curve had been obtained with known standards. The present work, designed for use in forward areas where surveys of nutrition are needed most, required that only the hardiest equipment be used. Fine electrical equipment would have been impractical and cumbersome under tropical field conditions. The method has been adapted for visual comparison which permits estimation of Vitamin C concentration to the nearest 0.05 milligram per hundred (100) cc of blood. This degree of accuracy is ample for practical purposes of usual survey work. More elaborate techniques permit closer comparison but the reliability or significance of the second decimal place in the measurement may be seriously questioned.

Visual depth colorimeters of the Klett type cannot be used in this method. The only practical means of color comparison is through the use of a graded series of color standards. These may be prepared by developing the 2:4 dinitrophenylhydrazone color in standard dehydroascorbic solutions in the same manner used for the treatment of protein-free filtrates from plasma or whole bloods. The standard dehydroascorbic acid may be obtained by charcoal treatment of standard Vitamin C solution. Such a color series must be prepared on each day that blood Vitamin C is to be determined. The red color in the standards is stable only a few hours and standard tubes cannot be saved from one day to the next. The usual practice in the pre-

sent work was to prepare the color series simultaneously with the determination of Vitamin C in ten (10) whole blood and ten (10) plasma samples.

The range of concentration in the color series is equivalent to blood Vitamin C of 0 to 1.2 milligrams per hundred (100) cc with stepwise differences of 0.1 milligrams per hundred (100) cc. When occasionally a higher blood Vitamin C is found, the color series must be extended into a higher range but this is not frequently necessary with normal troops in Manila.

The standard color series is prepared as follows:

(1) Prepare a standard Vitamin C solution, in five (5) per cent acetic acid, containing exactly 0.2 milligrams of ascorbic acid per cc. This stock standard is stable for two (2) weeks if kept in a refrigerator. The standard may lose reducing power during the 2-week period but the ascorbic acid plus dehydroascorbic acid content of the solution remains perfectly reliable for use as a standard in the color reaction. The standard Vitamin C solution may be prepared from pure crystalline Vitamin C or from standardized tablets containing exactly twenty-five or fifty (25 or 50) milligrams of ascorbic acid.

(2) Place six (6) cc of the standard ascorbic acid solution (containing 0.2 milligrams per cc) in a hundred (100) cc volumetric flask and dilute to the mark with five (5) per cent acetic acid. The solution now contains 1.2 milligrams of Vitamin C per hundred (100) cc.

(3) Place ten (10) cc of the solution (1.2 mgm Vitamin C per 100 cc) in a hundred and twenty-five (125) cc Erlenmeyer flask and add thirty (30) cc of trichloracetic acid and three (3) grams of activated charcoal. Mix vigorously and filter after the solution has been allowed five minutes contact with the charcoal. The filtrate now is at the

concentration, with respect to dehydroascorbic acid, found in charcoal-treated, protein-free filtrates of bloods containing 1.2 milligrams per hundred (100) cc. Three cc of this filtrate are then placed in a tube of the same size as those used for the treated blood and plasma filtrates. The tube is used for the color standard equivalent to 1.2 mg of Vitamin C per hundred (100) cc of blood. By correct dilution of portions of the Vitamin C filtrate (charcoal-treated) solution to three (3) cc, each of the other tubes in the graded series can be prepared. Color is then developed by adding thiourea solution and the 2:4 dinitrophenylhydrazine reagent to each tube in the series and carrying through the identical procedure described for the development of color in protein-free filtrates.

f. Visual comparison of "unknowns" with graded color series.

After development of the red color in the plasma and blood filtrates and in the graded color series, visual comparison is made by matching, as nearly as possible, the red cast of each unknown tube with a tube in the graded series. Interpolation to the closest half-step in the graded series is usually possible. Comparison is best made by viewing the solutions from the top of the tubes using a white surface for background. After some experience the comparison of degree of redness is not difficult. Whole blood filtrates frequently develop a slightly yellow cast but with proper conditions of lighting, differences in the red component are obvious and no difficulty is presented in judging concentrations with accuracy.

A special advantage of the Roe and Kuether method, over all the others used in blood Vitamin C estimation, is that the method measures dehydroascorbic acid. No special precautions are necessary for maintaining ascorbic

acid in the reduced form. Thus the method is hardy and well suited for field work.

5. Thiamine (Vitamin B_1) in Urine.

Estimation of thiamine in urine samples is based upon the quantitative conversion of thiamine to thiochrome by oxidation with alkaline potassium ferricyanide. Thiochrome is a fluorescent compound giving a blue color in ultra violet light, the intensity of which may be used as a means of measuring the thiamine concentration by visual means. Reference is made to the original source of this method; Wang Y. L. and Harris L. J., Biochemical Journal, 33, 1356, (1939). A few modifications have been introduced in adapting the method to conditions prevailing in tropical areas.

The technique used in the present work was developed by one of the officers during an assignment in Australia. Technical information and advice concerning the visual thiochrome method were obtained through:

University of Queensland Library, Brisbane.

Mr. S. Clive Graham, Gillespie Bros. Pty Ltd.
Pyrmont, Sidney.

Mr. C. A. Reid, Mauri Bros & Thompson, Ltd.
Waterloo, Sidney.

Mr. Farrer, Kraft Walker Cheese Co., Pty Ltd.
Melbourne.

Laboratory Staff, Royal Prince Henry Hospital
Long Bay, Sidney

a. Preliminary Extraction of Urine.

Place fifteen (15) cc of acidified urine in a Squibb type separatory funnel of fifty (50) cc capacity. Add an equal volume of isobutyl alcohol (Isobutyl alcohol used in this and subsequent steps must be freed of all

fluorescent materials by redistillation of isobutyl alcohol in an all-glass still. Contact with cork, rubber or waxes in distillation or subsequent storage must be avoided as they contribute fluorescent materials). Shake the funnel by gentle rocking and inversion for two minutes and then allow to stand until the isobutyl alcohol and urine layers separate sufficiently for the removal of at least ten (10) cc of extracted urine. (Stop cock grease may <u>not</u> be used in the Squibb funnel tap because of fluorescent contamination. Lubrication of stopcock with a drop of isobutyl alcohol is satisfactory).

Remove ten to twelve (10-12) cc of extracted urine into a second Squibb funnel and extract with ten (10) cc of "wet" isobutyl alcohol in the same manner ("wet" isobutyl alcohol is prepared by shaking isobutyl alcohol with water until complete saturation is reached). Allow the mixture to separate into layers until at least eight (8) cc of extracted urine can be drawn from the funnel. Place urine of the second extracting in a test tube in the direct sunlight for about one (1) hour.

Double extraction of urine with isobutyl alcohol serves to remove most of the interfering fluorescing substances from urine, including a large portion of the atabrine and products of atabrine metabolism. Subsequent exposure to direct sunlight destroys all of the remaining atabrine and other non-specific fluorescing substances. Sunlight intensity in Brisbane, Australia, (summer) and in Manila, P. I., is satisfactory for this type of treatment. It is not known whether sunlight would be as effective in other latitudes.

Preliminary extraction with "dry" isobutyl alcohol results in a five (5) per cent volume decrease in the urine sample. The volume loss occurs

as the result of mutual solubility relationships of water and isobutyl alcohol. No volume change occurs in the subsequent extraction with "we" isobutyl alcohol where water and alcohol phases are mutually saturated. Final results in this method must be corrected to allow for the volume change.

b. Oxidation with Alkaline Potassium Ferricyanide.

Place two (2) cc portions (exact measurement) of extracted urine in each of three glass-stoppered graduated cylinders of twenty-five (25) cc capacity. A fasting urine sample of this size, from normal subjects, contains 0.05 to 0.40 micrograms of thiamine. Subjects under vitamin therapy or during oral load tests produce urines of higher Vitamin B_1 concentration and special dilution must be made of such samples so that a two (2) cc portion does not contain an excessive amount of thiamine.

Add two (2) cc of methyl alcohol to each of the three cylinders and mix. Methyl alcohol exerts a protective and stabilizing effect during the alkaline oxidation of thiamine to thiochrome.

Add one (1) cc of thirty (30) per cent sodium hydroxide to the first two cylinders. The first cylinder will then serve as the "blank", unoxidized sample. The second cylinder serves only as a "control" sample for the oxidation reaction. After addition of alkali, add dropwise to the second cylinder, freshly prepared two (2) per cent, aqueous, potassium ferricyanide solution with continuous mixing and note the exact number of drops required for the sample to retain a ferricyanide yellow color for more than five (5) but less than fifteen (15) seconds. Comparison of the

color of the second ("control") cylinder with the first ("blank") facilitates judging of the ferricyanide end point. Accuracy in this step is requisite for a satisfactory estimation. After the end point is determined, the contents of the "control cylinder are discarded. (Excess ferricyanide is detrimental to the determination because it destroys some interfering non-specific fluorescing substances in the oxidized sample. The unoxidized sample will not serve as a valid blank for visual matching during the fluorometric titration if the oxidized tube has lost any of its interfering fluorescing substances).

Next add to the third cylinder the exact quantity of 2 per cent ferricyanide solution determined as essential for oxidation of the "control " solution (second cylinder). After thorough mixing, add one (1) cc of thirty (30) per cent sodium hydroxide solution. Mix the solution and let stand for thirty (30) seconds before proceeding with the isobutyl alcohol extraction described below.

c. Extraction of thiochrome with isobutyl alcohol.

Add ten (10) cc of isobutyl alcohol to the unoxidized blank solution (first cylinder) and to the oxidized solution (third cylinder). Mix the contents for two (2) minutes by gentle rocking and inversion of the cylinders. During this procedure thiochrome and other non-specific fluorescing substances pass into the alcohol phase. Allow the contents to separate into phases and remove the water layer(bottom) from each cylinder by means of a fine capillary pipette provided with a rubber suction bulb ($\frac{1}{4}$ oz capacity).

Both samples are then washed by shaking for twenty (20) seconds with four (4) cc of distilled water. After settling, remove the water phase with the capillary pipette.

Dilute both the blank and oxidized samples to exactly fifteen (15) cc with isobutyl alcohol and mix each thoroughly. Remove ten (10) cc of each of the samples (two-thirds of the original extracted samples) by means of a volumetric pipette and place them in each of two identical and optically matched test tubes of twenty (20) cc capacity (soda glass tubes are satisfactory or pyrex test tubes containing practically no fluorescing materials may be used). To each sample, add one (1) cc of absolute alcohol and mix. The alcohol makes all traces of residual water droplets completely soluble, thus clearing the samples for the visual fluorometric titration which follows.

d. Fluorometric titration (visual).

(1) A source of ultra violet light is the only special equipment needed in the fluorometric titration. Two types of arrangement have been employed in the present work with equal success.

(a) In one arrangement, an Australian General Electric mercury vapor bulb of a hundred and twenty-five (125) watt capacity was used. This bulb (Phillips Phillora) is supplied by the manufacturer encased in a large outer bulb of colbalt glass which serves to screen out most of the visible light. The bulb operates on 220 Volt circuit and must be provided with a suitable electric choke. The bulb and housing are best installed in a room that can be completely darkened, making fluorescent colors clearly visible during titration. A satisfactory housing for the bulb may be built of ply wood and painted black inside to prevent reflaction of any visible light. The box-shaped housing should be open on the front side so that tubes, during titration, can

be held in the light beam. However, the open side of the box should be provided with a plywood screen covering the top half of the open side. This is essential to protect the operator from looking directly at the strong ultra violet light source. Severe eye injury results from over-exposure to ultraviolet light and sometimes occurs before the delayed symptoms of ultraviolet burn become apparent.

b. Another satisfactory arrangement was constructed with an American General Electric Type #4 mercury vapor bulb provided with an air cooled metal housing and a Woods' glass screen to filter out most of the visable light. The installation operates on 110 volt circuit and requires an autotransformer (General Electric Type 59G 18). In the present work, this apparatus has been used in the regular lighted laboratory. The horizontal light beam of ultra violet is directed into an otherwise light tight box which is provided with holders in which the pair of tubes for comparison stand vertically in the ultra violet beam. The light tight box is provided with an eye slit at the top for viewing the tubes.

(2) Thiochrome Standards.

Thiochrome standard solutions are prepared by oxidation of a standard thiamine hydrochloride solution. The most convenient thiamine standard is one containing fifteen (15) micrograms per cc in N/10 (tenth-normal) hydrochloric acid. Such a standard may be prepared from pure thiamine hydrochloride, standardized ampoules of thiamine hydrochloride solution for injection, or standardized thiamine hydrochloride tablets of known content. Thiamine standards in

hydrochloric acid are stable for months without refrigeration.

The oxidation of standard thiamine solution to obtain standard thiochrome is in every way identical with that used in the oxidation of urine samples except that only one (1) drop of two (2) per cent potassium ferricyanide is used. After extraction of the thiochrome with isobutyl alcohol, the extract is diluted with enough isobutyl alcohol to produce the desired strength. In the present work, it has been found convenient to use two thiochrome standards; one, used in the titration of fasting urine samples, was equivalent to 0.2 micrograms of thiamine per cc; the other, used in titration of load test urine samples, was equivalent to 0.5 micrograms of thiamine per cc. Thiochrome standards must be prepared fresh daily and protected from direct light.

(3) Microtitration.

Hold the tubes containing blank and oxidized samples vertically in the horizontal ultra violet light beam, and view the tubes from the top. The oxidized tube, with thiochrome fully developed, yields a blue fluorescence and the blank tube a faint silver color. Make stepwise additions of standard thiochrome solution to the blank sample and mix after each addtion. View the tubes side by side and note the blue color of the blank solution approach the intensity of color in the oxidized sample as the titration proceeds. As the end point is approached, stepwise addition of thiamine standard is cut to 0.05 cc. The end point is judged when the blank tube attains a blue color identical with the oxidized tube. Stepwise addition of standard thiochrome to the blank tube is made by means of a 0.25 cc graduated serological pipette. As the quantity of solution in the blank tube

becomes greater during titration, due to addition of standard thiochrome, an equivalent amount of isobutyl alcohol is added to the oxidized tube to maintain identical volumes throughout.

e. General Comment.

The accuracy and reliability of this method has been tested by Wang and Harris (the originators) and by many others including: Pyke, M, Journal of the Society of Chemical Industry, 58, 338 (1939) and Slater, E. C., Australian Journal of Experimental Biology and Medical Science 19, 29, (1941).

After some experience has been gained, the method becomes simple in operation. The practical nature of the technique and its simplicity make a strong recommendation of the method for field work. Visual fluorometric titration avoids the use of complicated electrical apparatus which has no practical place in forward areas where army nutrition surveys are of special importance.

6. Riboflavin (Vitamin B_2) in Urine.

a. General Comments.

The method for determining reboflavin in urine depends on measurement of the yellowish green fluorescence of this vitamin in ultra violet light. The method has been adapted, for use in field conditons, to the visual fluorometric titration technique and is based upon the "direct" method of Najjar. The original procedure employs the fluorophotometer for making the measurements, a technique impractical for the present work. Reference is made to Najjar V. A., Journal of Biological Chemistry 141, 355 (1941).

The modified method described below is capable of reasonable accuracy

and the reliability has been checked by analysis of aqueous riboflavin solutions of known strength and by recovery, from urine, of added known amounts of riboflavin. Recoveries of ninety to ninety-seven per cent were found.

Atabrine and its end products in urine have been a serious problem in riboflavin estimations in the Pacific theater. Atabrine has a yellow fluorescence so like that of riboflavin that the compound cannot be differentiated visually. Similarity of structure and chemical behavior of atabrine and riboflavin limits the methods of separating them. Solution of the atabrine problem resulted when it was found, in this laboratory, that permanganate oxidation of a more vigorous character, than that used originally by Najjar, will completely destroy atabrine and leave Vitamin B_2 unaffected.

Urine samples to be analyzed must be protected from direct sunlight which very rapidly destroys riboflavin. Analysis is completed on the day the urine sample is collected, and all procedures are carried out in diffuse light as rapidly as possible to avoid loss due to exposure to light. In the tropics the penetration of rays destructive to riboflavin is intense; even too bright diffuse light may seriously interfere with the accuracy of the method.

b. Destruction of atabrine and other interfering fluorescing substances.

Place two (2) cc of glacial acetic acid and four (4) cc of pyridine in a fifty (50) cc graduated glass-stoppered cylinder and mix. Add ten (10) cc of urine (when load test urine samples are examined use four (4) cc of urine and six (6) cc of water) to the mixture and enough four (4) per cent aqueous potassium permanganate to produce a distinct purple

color in the solution for at least 3 minutes. Two to three (2-3) cc of permanganate are usually required for ten (10) cc of urine. Much of the permanganate is converted rapidly into the brown oxide especially in more concentrated urines. An excess of oxidizing agent (permanganate) must be established by maintaining a purple color for the specified time (3 minutes) to produce complete destruction of atabrine. Allow the oxidation to proceed for a total of fifteen minutes and add more permanganate solution (after 3 minutes) only if the precipitate of brown oxide lightens perceptibly in color. At the end of fifteen minutes add three (3) per cent hydrogen peroxide solution dropwise until the mixture is clear yellow in color.

c. **Extraction of riboflavin with isobutyl alcohol.**

Add twenty (20) cc of redistilled isobutyl alcohol and ten (10) grams of anhydrous sodium sulfate to the mixture in the cylinder. Stopper the cylinder and mix thoroughly by shaking for two minutes. Set the cylinder in a dark cupboard for five or ten minutes to allow separation into layers while protected from light. During this procedure, all of the riboflavin and most of the pyridine and acetic acid pass into the isobutyl alcohol phase. Extraction is facilitated by the sodium sulfate which reduces the solubility of these compounds in the water phase by a "salting-out effect".

Remove two ten (10) cc samples of the extract (isobutyl alcohol phase) with a volumetric pipette and place them in each of two identical and optically matched test tubes of about twenty (20) cc capacity. Add to each portion one (1) cc of absolute ethyl alcohol to clear the solution of any cloud due to suspended water droplets.

Place one of the tubes in a dark cupboard for protection from light until the second tube is treated according to the following procedure.

d. Preparation of riboflavin blank by exposure to sunlight.

Place the second tube out of doors in the direct sunlight for complete destruction of the riboflavin. In Manila during July and August destruction is complete in ten to fifteen minutes of direct exposure. Even with a fairly heavy mist layer and weak sunlight penetration, destruction seldom requires more than thirty to forty-five minutes in these latitudes. (Exposure of solutions to ultraviolet lamp of 125 Watt Capacity for a half-hour results in little loss of riboflavin). Completeness of riboflavin destruction is determined by observing the tube in ultra violet light for complete absence of yellow fluorescence. When fading is complete, the solution serves as the riboflavin "blank" for the fluorometric titration which follows.

e. Riboflavin standards for titration.

Riboflavin standards may be prepared from pure riboflavin or standardized riboflavin tablets containing one (1) milligram of the vitamin. Such solutions are made by dissolving the riboflavin in distilled water and taking up the correct quantity of the water solution in isobutyl alcohol with enough ethyl alcohol added to give a clear solution free of water droplets. The two standard strengths used in the present work were: two (2) micrograms of riboflavin per cc, for urines from fasting subjects, and ten (10) micrograms per cc, for titration of samples after load test doses of riboflavin.

f. Fluorometric titration.

Titrate with the same technique described previously for thiochrome estimations, using the sunlight-faded tube as the blank to which standard riboflavin solution is added. Titration end point is reached when

the blank tube attains the same yellow fluorescence as the tube which was not exposed to sunlight.

g. Note on the Calculation.

When ten (10) cc of acidified urine are treated in the manner described above and extracted with twenty (20) cc of isobutyl alcohol, the final volume of riboflavin extract (containing isobutyl alcohol, pyridine acetic acid and some water) is 27.5 cc. Ten cc portions of the extract were taken for the blank and unfaded tubes. Thus, the unfaded tube contained an extract from $\frac{10}{27.5} \times 10 = 3.64$ cc of the original urine. This volume relationship must be used in the calculation of riboflavin content of the original sample.

Inclosure 5

Results and Interpretations

Part I - Study of the Whole Experimental Group.

1. General Comment.

The observations on nutritional status, which have been obtained through the application of a diverse array of physical and metabolic criteria, are presented in detail in this and subsequent inclosures. The study is unique in the thoroughness with which it evaluates the nutritional condition of a representative group of American soldiers in the tropical service. The series of data has been studied for correlations existing within it and where correlations have been found, they may indicate the operation of fundamental nutritional principles. However, the study was limited to one hundred and eleven (111) subjects and therefore one cannot entirely rule out the possibility of some correlations arising through chance or coincidence. Therefore, rigid interpretation of the study is not intended and the data are viewed as descriptive of a group rather than as proof of general nutritional theories.

The work is reported in the belief that it demonstrates the potential value of future experimentation along similar lines. Such research should result in a fuller knowledge of the relation of nutrition to health and the effect of subclinical nutritional deficiency upon the well-being of troops.

2. Characteristics of the Experimental Group.

All of the subjects chosen for observation were on full duty status with no abnormalities apparent to themselves or their commanding officers. Five of the subjects were officer personnel and the rest (106 subjects) were from enlisted ranks.

The sections which follow are designed for presentation of a large body of data. The interpretations are based upon distribution tables and charts contained in Inclosure 8. In the text, distribution of values is described through consideration of the average for each series of data and the extreme limits of the observed values. In addition, the "mode" and "median" have been determined for each series of data. These terms are used throughout the text. The mode and median values in each series frequently differ from each other and from the average, and existence of such differences shows skew qualities in some of the data. (The "mode" in a series of data is that point in the range of values which includes the largest number of subjects. Thus the mode of age is that age group containing more subjects than any other age group in the whole series. The "median" is that point in the range of values which exactly divides the experimental group in half. Median of age, for example, is that age with respect to which, exactly 50 per cent of the subjects are younger and 50 per cent are older).

a. Age of subject.

Subjects in the experimental group ranged in age from 19 to 39 years and the average age was 29.2 years. The greatest number of subjects in any age group were in the group from 24 to 26 years (mode). Half of the subjects were 25 or more years old (median). Reference is made to Table 1 (Inclosure 8) for full presentation of age distribution.

b. Height and weight.

Height of the experimental troops showed extremes of 61 inches (5 ft, 10 inches). Mode of height was 70 inches and median 69 inches.

Weight extremes were 100 pounds and 245 pounds. The average was 159 with mode and median at 150 to 160 pounds.

Body contour depends upon both height and weight. Rough comparison of the subjects as to stoutness of body build may be obtained by dividing weight in pounds by height in inches, the weight-height ratio. The weight-height ratios ranged from 1.6 to 3.5 with average mode and median at 2.3.

Comparison of these data with standard weight and height tables shows no significant tendency of distribution toward fatness or leanness in the experimental group. As regards height and weight, the subjects appear identical with any similar group which might be selected in the United States. Reference is made to Tables 2 and 3 (Inclosure 8) for detailed analysis of the height and weight distributions and the weight-height ratio.

c. Length of military service and service overseas.

The length of military service varied from 10 to 66 months with an average of 29 months. Service overseas ranged from 1 to 31 months and the average was 13 months. Practically all of the overseas service was in tropical areas.

d. Activity of subjects.

No really satisfactory measure of activity of the experimental troops was available. The subjects were selected from a variety of service units and degree of activity undoubtedly varied. For purposes of description, the group has been divided roughly into the three classifications of heavy, light and medium activities. Degrees of activity have been judged on the basis of type of duty to which each subject was assigned in his unit. The "heavy activity group" contained heavy construction crews, longshoremen and heavy equipment mechanics. The "medium activity group" consisted of truck drivers, small arms and equipment repairmen and military police.

In the "light activity class" were clerks and supervisors, mess personnel, sedentary technicians, gun watch crews and jeep drivers.

Of all subjects, 52 were classed as performing light activity, 34 medium and 25 heavy activity.

e. Medication.

All subjects were questioned concerning routine taking of atabrine. Ninety-four subjects stated that they took one atabrine daily, 3 took atabrine irregularly and 14 took none. Experience in the laboratory with urine analysis indicated that the information given on atabrine medication was not entirely true. It is doubtful whether more than half the group took any atabrine. There may have been some fear on the part of the subjects that admission of not taking atabrine would result in disciplinary measures inasmuch as atabrine medication is commanded by specific order.

No subject in the experimental group had taken any vitamin preparation in the 6 months prior to the experiment.

f. Morale and appetite.

During the study each subject was given a questionaire in which he was requested to make a statement concerning his appetite and state of morale. The subjects were assured that the questionaire was confidential and that any unsatisfactory rating would not lead to further questioning. The subjects were told to rate these matters accordingly to their own judgment classing morale and appetite as excellent, good, fair or poor. Such ratings are subject to many complicating factors and thorough interpretation cannot be made. However, it is believed that some indication of mental attitudes and sense of well-being results from this sort of questioning. It was generally true that the subjects were interested

in participating in the study and it appeared that they rated themselves in good faith and with a willingness to cooperate.

The use of a confidential questionaire seems more informative than direct verbal questioning. During clinical examinations, reported in Inclosure 7, the subjects were asked by examining officers about appetite and morale. Under these circumstances the subjects gave almost universally "satisfactory" answers. The answer "good" seemed to be more or less automatic in the subjects and probably was used as defense against further inquiry into personal business. The mild mental stress of subject during physical examination, in a state of seminudity, is not likely to favor a thorough consideration, by each subject, of his own personal attitudes. Furthermore, personal pride might dictate a satisfactory rating to avoid being classified as unable to "take it" overseas. For these reasons, only the ratings obtained in questionaires have been used in interpretations and the ratings obtained in clinical examination have been entirely omitted.

In judging morale, 4 subjects rated themselves excellent, 38 good, 46 fair and 23 poor. In judging appetite, 5 subjebts rated themselves excellent, 44 good, 43 fair and 19 poor. Reference is made to Tables 16 and 17 and charts 1 and 2 in which the ratings are presented fully.

g. Medical histories.

Medical histories were obtained on all subjects by means of questionaires. Several sources of error operate in the questionaires and these must be recognized during interpretations: (1) subjects may classify disorders incorrectly or (2) hypochondria or desire to complain may color the response of some subjects. In a later part of the study, when

clinical examinations were given, medical histories of a more reliable type were obtained and will be reported in Inclosure 7. The histories considered here are based on the subjects' own statements of disorders occurring within the 6 months period prior to the experiment.

Sixty-five subjects reported no disorders within the past 6 months and 36 reported one or more disorders. Of those who reported disease there were 17 skin disorders of all types (heat rash was not included) such as acne, impetigo, folliculitis and epidermophytoses, 15 acute intestinal disturbances reported from 1 to 10 episodes during the period under consideration. Some had received medical care for diarrhea but it is not known how many received sulfaguanidine therapy (which through its effect on intestinal flora may considerably alter nutritional state in the Vitamin B-complex). Reference is made to Table 18 for summary of these results.

h. Character of the dietary at the time of the study.

Ration surveys were made by 1st Lt Joseph Weybrew, Sn C, Nutrition Officer, Base X, during the time biochemical studies were under way. The surveys included 6 of the 11 units from which subjects had been chosen. Ration analysis was made on the basis of records on rations drawn during a two or three weeks period. Evaluation of allowance of specific nutrient factors was made in accordance with the usual army practice based on quantitative allowance of several food groups. Standard nutrient values were applied to the food group allowances per man per day to secure an estimate of the daily provision of specific minerals and vitamins. The table of nutrients provided per man per day is presented below:

Nutrient	Average Provision in 6 units	Recommended* Allowance
	(per man per day)	
Calories	4520	3500
Protein (gm)	150	120
Fat (gm)	200	-
Carbohydrate (gm)	530	-
Calcium (mgm)	830	800
Phosphorus (mgm)	2100	-
Iron (mgm)	27	12
Vitamin A (International Units)	8700	5000
Thiamine (mgm) (Vitamin B_1)	1.9	1.6
Riboflavin (mgm) (Vitamin B_2)	2.7	2.2
Nicotinic Acid (mgm)	32	18.0
Ascorbic Acid (mgm) (Vitamin C)	73	75

*Recommended daily allowance of nutrients for moderate activity, Committee on Food and Nutrition, National Research Council, (1945).

The ration analysis demonstrates that there was no lack of nutrients in the ration drawn by participating units. All nutrients were provided in such quantity that the ration exceeds or comes close enough to recommended levels to insure a reasonable factor of safety in group feeding.

Therefore it was possible to predict that no full duty troops in the Manila area could develop any obvious clinical signs of malnutrition.

Calorie allowance of 4100 to 5200 per man per day seems very high. The best authority recommends 3500 calories for moderate activity in temperate climates. Accomodation to tropical conditions and sedentary activity is believed to further reduce caloric requirements. Thus it appears that the ration would permit many soldiers to fail in consumption of a large portion of their food allowance. Sedentary individuals might choose from the ration only those items dictated by individual taste and dietary habits. It is apparent that dietaries as consumed may be more or less satisfactory in nutrient value than the whole ration depending upon the nutrient value of foods selected by each individual.

A ration evaluation is an approximate indication of the nutritional soundness of the ration but fails entirely as an indication of the nutritional benefit accruing to each individual presented with the ration. As a matter of fact, the present study reveals a considerable variation in nutritional state among a group of subjects receiving a ration judged as nutritionally adequate. There are factors involved in nutritional state other than provision of a theoretically adequate dietary and there is no assurance that such a ration in itself will produce perfect nutrition in every individual to whom the ration is issued.

3. Basis for Interpretation of Biochemical Tests.

The results of biochemical tests applied to the experimental group are presented and interpreted below in accordance with:

(a) Report #19, Harvard Fatigue Laboratory to OQMG, "The Nutritional status of enlisted Men in the Desert Training Center Area", by Johnson, Sargent, Robinson and Consolozio, dated 15 Oct. 1943.

(b) Project #30 by Armored Medical Research Laboratory, Fort Knox, Ky to O.Q.M.G. dated 22 Nov 1944.

The cited reports have established the lower levels of performance, in biochemical tests, of men considered normal in physical fitness, morale and general health; and have also established those levels of performance which suggest deficiency severe enough to impair morale and physical fitness. These levels were obtained with men under extreme conditions and are the best available criteria on which to base interpretation of biochemical data relating to nutritional state. The levels of response in biochemical tests giving presumptive evidence of deficiency are given, for reference, in Table 4 (Inclosure 8) and will be constantly referred to in the discussion which ensues.

4. Chloride Ion Excretion.

The urinary output of chloride was measured in 110 fasting subjects and the results are expressed as grams of sodium chloride excreted per fasting hour. Interpretation of the results (authority cited above) was made on the basis that urinary excretion values less than 0.2 grams of sodium chloride per fasting hour indicate "severe" depletion of salt. In temperate areas excretions of above 0.5 grams per hour are usual and 0.8 to 0.9 grams are not uncommon.

Sodium chloride excretions by the experimental subjects during fasting are presented fully in Table 5 and Chart 5 (Inclosure 8). The extreme range was from 0.02 to 0.95 grams of urinary sodium chloride per fasting hour with the average at 0.36 grams. Mode and median were lower than average, falling between 0.21 and 0.31 grams. Twenty-eight subjects excreted less than 0.20 grams of sodium chloride per fasting hour and seven of these excreted less than 0.10 grams. Therefore, 25 per cent of the experimental group could be

classed as showing severe deficiency with respect to sodium chloride and many more subjects excreted but little in excess the minimal normal quantity.

The findings are consistent with current theories relating to the effect of tropical environment upon chloride economy in the body. Loss of sodium chloride may be severe in profuse sweating where heat is intense as in Manila during the period of study (July and August). Only 20 per cent of the subjects showed urinary excretions of sodium chloride per fasting hour in excess of 0.50 grams. Under controlled conditions, it is possible to maintain health in experimental animals with dietaries so low in sodium chloride content that urinary chloride excretion falls to zero. Sodium chloride balance is maintained in such animals through a very efficient retention of body salt. In the active human subject, the situation is quite different. Where the sodium chloride supply is already low and sweating supervenes with its attendant sodium chloride loss, rapid development of deficiency in electrolytes of the body may be expected, with undesirable physiological effects of dehydration and "heat cramps". Some scientific evidence supports the theory that the body has a limited ability to accomodate to tropical environment and that after acclimatization, salt content of sweat may be reduced, thereby assisting in conservation of chloride to some extent.

The fact that many subjects in the present study have very low fasting urinary excretions of sodium chloride supports the belief that in tropical messing, the correct seasoning of food with salt is an important matter and that the offering of salt tablets to troops during heavy work and at meals is wise practice.

5. Plasma Protein and Blood Hemoglobin Concentrations.

a. Plasma protein.

During the study, plasma protein concentration was determined in 109 of the subjects. Plasma protein is considered normal in any concentration between the minimum of 5.8 grams per 100 cc of plasma and the upper limit of 7.0 to 8.0 grams. When plasma protein falls to 5.2 or less, symptoms of edema are almost invariable. Hypoproteinemia is associated with all disease states causing a loss of plasma protein (as nephritis) or any disorder preventing adequately rapid synthesis of plasma protein. Where none of these disease conditions exist, hypoproteinemia is strongly suggestive of an inadequate dietary intake of protein.

The experimental group (109 subjects) had plasma protein concentrations ranging from 5.8 to 7.6 grams per 100 cc with the average at 6.7. Mode and median appeared in the range of 6.5 to 6.8 grams of protein per 100 cc of plasma. Only one subject had as little as the minimal normal concentration of 5.8 grams per cent plasma protein and 17 subjects had concentrations in excess of 7.0. Complete details of plasma protein findings are given in Table 6 (Inclosure 8).

The results give presumptive evidence that protein nutrition of the subjects was good. It is not known whether any of the high concentration (above 7.0 grams per cent) of plasma protein might be associated with dehydration (hemoconcentration). The higher levels of plasma protein were not consistently associated with low fasting urinary excretion of sodium chloride.

b. Hemoglobin content of blood.

Hemoglobin concentration was determined in 107 of the subjects. Normal hemoglobin concentration is generally accepted as lying in the range of 15 to 19 grams per 100 cc of blood, but there is a growing belief that true normalcy has a more restricted range and that slight lowering of hemoglobin,

even within so-called "normal limits ", may be of greater significance than is known at present. "Serious" deficiency in blood hemoglobin is evidenced by concentration of less than 12 grams per cent.

The extreme range of hemoglobin concentration in 107 subjects was from 12.8 to 20.1 grams per cent with the average and median at 16.0. Distribution of the values was bimodal with maxina occurring at 15.4 and 16.6 grams per cent. Full presentation of the hemoglobin data has been made in Table 7 and Chart 3 (Inclosure 8).

Seventeen subjects had blood hemoglobin concentrations below the normal minimum of 15 grams per cent but none had a "serious" deficiency concentration below 12 grams per cent. Two subjects showed hemoglobin concentrations in excess the normal maximum of 19.0.

The study reveals no serious shortage of hemoglobin or marked excess. There is no way of determining how much effect tropical environment with possible tendency to hemoconcentration has resulted in raising the general level of hemoglobin concentration in this particular group. The fact that no apparent inverse correlation exists between hemoglobin level and sodium chloride excretion in fasting urine does not entirely eliminate the possibility that hemoconcentration may be operative in some of the subjects.

In connection with possible hemoconcentration, it is of interest to note that two subjects having 19.5 and 20.1 grams per cent hemoglobin (in excess of the normal maximum) excreted 0.07 and 0.14 grams of urinary sodium chloride per fasting hour (less than the normal minimum). Low excretion of sodium chloride was not invariably associated with high hemoglobin levels. Nevertheless these two instances offer suggestive evidence

that hemoconcentration associated with sodium chloride loss and consequent distortion of body water balance may influence the over all picture of hemoglobin concentration of subject in tropical areas.

6. Vitamin C Excretion in Urine During Fasting.

Excretion of Vitamin C in urine during fasting was determined in 100 of the subjects. Less than 0.30 milligrams of ascorbic acid in urine per fasting hour is presumptive evidence of Vitamin C depletion according to authority cited above. Urinary excretions between 0.30 and 1.0 milligrams per fasting hour are considered within the normal range.

The 100 experimental subjects exhibited extreme ranges of urinary excretion of 0.10 to 1.80 milligrams of ascorbic acid per hour with the average at 0.54 and median at 0.50. The mode (considerably lower than the average), being in the range of 0.31 to 0.40 milligrams per fasting hour, shows a general tendency of the group to low urinary excretion of ascorbic acid. Reference is made to Table 8 and Chart 5 (Inclosure 8) for complete data on the fasting urinary Vitamin C study.

Nine subjects excreted less than 0.20 milligrams of Vitamin C in urine per hour and were well within the range suggesting depletion. Blood Vitamin C studies (reported below) do not bear out the theory that these nine subjects were approaching depletion since their blood Vitamin C concentrations averaged 0.51 milligrams per 100 cc which is well above depletion levels and not substantially below the average for the whole group (0.61 mgm per cent). It is concluded that, in the hands of the present investigators, the fasting excretion of urinary ascorbic acid fails to test state of body saturation with respect to Vitamin C. The results are consitent with reports by other workers relative to the subject.

Although low excretion of Vitamin C was not a reliable indication of poor body saturation, it appears that very high Vitamin C fasting excretions were generally associated with higher blood levels. Thus, seven subjects in the group, excreting more than 1.00 milligrams of ascorbic acid per fasting hour (normal maximum), had high average blood levels of 0.78 milligrams per cent Vitamin C. However, not all high blood levels are associated with high excretion of urinary Vitamin C during fasting.

It seems likely that there are many factors which vitiate the use of fasting urinary ascorbic acid excretion as an index of state of body saturation with respect to Vitamin C. Blood level is not the only determining factor in Vitamin C excretion, and only in extreme ranges of Vitamin C excretion is any relation with blood level evident. Failure of relation of fasting excretion to body saturation (as measured by blood level) in the case of Vitamin C is consistent with the findings (reported below) on general failure of relation between fasting excretion of Vitamin B_1 or B_2 and excretion of these vitamins after oral test doses of these vitamins (measure of body saturation).

7. Vitamin C Concentration in Whole Blood and Plasma.

Vitamin C was determined in both whole blood and plasma in 38 of the subjects, in plasma only in 32 subjects and in whole blood only in 31 subjects. Thus, of an experimental group of 99 subjects, there were 69 examinations for whole blood Vitamin C and 70 examinations for plasma Vitamin C. The method used, estimates ascorbic acid plus the small amount of dehydroascorbic acid which is present and these together constitute the blood or plasma Vitamin C concentrations referred to in the present discussion.

The 69 blood Vitamin C estimations showed an average concentration of 0.63 milligrams of Vitamin C per 100 cc of whole blood. From 70 estimations on plasma the average was 0.56 milligrams per cent.

In the 38 subjects examined for both plasma and whole blood concentration of Vitamin C, it was found that whole blood values were very consistently 0.05 milligrams per cent higher than plasma values. The difference is undoubtedly due to the high concentration of Vitamin C present in the white blood corpuscle fraction, which is frequently 20 to 30 times as great as that of the plasma. With so consistent a difference between plasma and whole blood Vitamin C, it has been convenient to correct plasma Vitamin C by adding 0.05 milligrams per cent to obtain the approximate whole blood Vitamin C values in the 32 subjects for whom only plasma values were originally obtained. It has thus become possible, in the interests of brevity, for the whole series of 99 subjects to be interpreted together on the basis of whole blood levels of Vitamin C. If the 69 plasma values and 70 whole blood values were interpreted separately, the findings would be essentially the same.

It is generally believed that plasma Vitamin C concentration below 0.40 milligrams per cent indicates a partial desaturation of tissues with respect to ascorbic acid not consistent with optimal nutrition. By inference one might assign 0.45 milligrams per cent Vitamin C as the critical level for whole blood. In the present discussion whole blood Vitamin levels of 0.40 milligrams per cent or less will be assumed to indicate partial desaturation and levels of 0.45 milligrams per cent or more to indicate satisfactory condition with respect to Vitamin C.

For the whole group of 99 subjects used in the work, the range in whole blood Vitamin C concentration was 0.25 to 1.60 milligrams per cent with an average of 0.61. The average was well above the critical level of 0.45 mil-

ligrams per cent. Mode and median appeared close to 0.55 milligrams per cent. The lowest value in the series of 0.25 milligrams of Vitamin C per 100 cc of whole blood, while not believed consistent with optimal nutrition, is not sufficiently low to suggest danger frank scurvy. Reference is made to Table 9 and Chart 5 (Inclosure 8) in which the blood Vitamin C data are fully described.

Twenty-five subjects had whole blood Vitamin C levels in the "desaturation" zone of 0.40 milligrams per cent or less. Twenty-four subjects had blood levels above 0.70 and may be considered as well saturated in Vitamin C. The 25 subjects who were judged as being partially desaturated had an average urinary ascorbic acid output of 0.44 milligrams per fasting hour compared with 0.54 milligrams per fasting hour which was the average for the 24 subjects showing good saturation according to the blood tests. The difference between the "critical" and "excellent" groups is so small that emphasis is given to the belief that fasting urinary excretion fails as a measure of Vitamin C status of human subjects. Determination of whole blood or plasma concentration remains the method of choice in evaluating Vitamin C nutrition.

8. Vitamin B_1 (Thiamine) Excfetion in Urine During Fasting.

Thiamine excretion was measured in 110 of the subjects during fasting. Normal performance in fasting urinary excretion of thiamine is evidenced, according to authorities (cited above), by an output of 2 micrograms or more per hour. The upper limit of normalcy is stated as 25 micrograms per fasting hour.

The extreme range of urinary excretion observed in the experimental subjects was 0.4 to 22.5 micrograms of thiamine per fasting hour with an average at 5.6 micrograms. The median was 5.0 micrograms and the mode 4.1 showing a definite tendency toward skewness in the direction of the lower end of the

normal range. Complete details of the urinary fasting thiamine data are given in Table 1 and Chart 4 (Inclosure 8).

Six of the 110 subjects excreted less than 2 micrograms of thiamine per fasting hour and according to the current interpretation this is evidence of desaturation of a severe degree. Ten subjects excreted more than 10 micrograms of urinary thiamine per fasting hour showing excellent saturation with this vitamin.

9. Vitamin B_1 Excretion in Urine After Oral Test Dose (5 milligrams of thiamine).

The urinary excretion response over a 4-hour period following oral administration of 5 milligrams of thiamine was measured in 106 of the experimental subjects. The "load" test was administered with the breakfast meal and has special significance as a measure of state of saturation of the subjects with thiamine. The rationale behind this load test is that low excretions of the test dose demonstrate excessive retention and give presumptive evidence that the organism is in need of the vitamin; likewise, high excretions of the test dose give evidence of an excellent degree of saturation.

According to the authority used in these interpretations (cited above), 50 micrograms of thiamine excreted in urine over the 4-hour period subsequent to ingestion of the test dose marks the minimal normal range. The maximum is stated to be about 800 micrograms during the 4-hour test. Excretion below 50 micrograms is presumptive evidence of severe depletion.

In the present series of subjects the range of performance on the load test was 21 to 780 micrograms of urinary thiamine per 4 hours with the average at 160 micrograms. The median was at 150 and skewness of the data in the direction of lower levels of performance is evidenced by the mode appearing between 50 and 100 micrograms of urinary thiamine per 4 hours. The general behavior in the

data is consistent with that observed in the fasting thiamine test. Complete details on the thiamine load test data are given in Table 11 and Chart 4 (Inclosure 8).

The average load test response in this group of 160 micrograms is of interest when compared with the average response of 400 to 500 micrograms of thiamine made by 600 representative normal troops in the Armored Medical Laboratory tests carried out in Colorado. The discrepancy in performance of the Manila soldiers compared with that of normal troops in the United States supports the belief that troops in tropical service are in a lower plane of nutrition generally than are "stateside" troops. (Lowered plane of nutrition does not infer "deficiency"). Further evidence of lowered plane of nutrition in the Manila group is the average fasting urinary excretion of 5.6 compared with 12 micrograms per hour as an average fasting performance in the Colorado tests.

Ten subjects made thiamine load test excretions of less than 50 micrograms and were classified as seriously depleted. Only 10 subjects excreted more than 300 micrograms in the 4-hour thiamine load test (performance in the order of the average for normal troops in the Colorado tests).

A low degree of correlation exists between fasting and load test performance with respect to thiamine. The correlation becomes clearer when only extremely high and extremely low urinary responses are considered. For example: Ten subjects, who made a high urinary response to the thiamine load test by excreting an average of 470 micrograms in 4 hours, excreted on the average 8.8 micrograms of thiamine per fasting hour. On the other hand, 10 subjects who showed evidence of desaturation or borderline performance with an average excretion of 35 micrograms in the thiamine load test made an average fasting excretion of 2.8 micrograms of thiamine per 4 hours.

In spite of such evidence of correlation, the interpretation of severe desaturation on the basis of fasting thiamine excretion leaves much to be desired. Thus, the group of 10 subjects, who evidenced severe depletion in the thiamine load test, contained only 3 of the 6 subjects judged as severely depleted according to the fasting excretion of thiamine. Furthermore, 2 of the 10 subjects judged as depleted on the basis of load test made fasting thiamine excretions as high or higher than those giving high responses to the load test.

The load test is very definitely the method of choice in the hands of the present investigators for determining state of body reserves with respect to thiamine. The discrepancy between fasting and load test responses in the thiamine study are reminiscent of the discrepancy between fasting urinary excretion of Vitamin C and state of body reserves judged on the basis of blood concentration of Vitamin C. Apparently there are complex factors influencing the fasting excretion which tend to vitiate the fasting excretion value as a reliable measure of the state of thiamine saturation of the subject.

10. Vitamin B_2 (Riboflavin) Excretion in Urine During Fasting.

Fasting excretion of riboflavin was measured in 101 of the subjects. According to authorities (cited above) the range of normal urinary excretion of riboflavin during fasting is 10 to 100 micrograms per hour. Fasting excretions below 10 micrograms per hour indicate desaturation in severe degree.

The group of experimental subjects excreted from less than 2 (the lower limits of the method used) to 80 micrograms of urinary riboflavin per fasting hour. The average excretion for the group was 11.2 micrograms and the mode and median appeared in the neighborhood of 9 micrograms per fasting hour.

Complete distribution of the fasting riboflavin excretion data is presented in Table 12 and Chart 4.

Sixty-three per cent of the subjects gave fasting urinary excretions of riboflavin below 10 micrograms per hour and show presumptive evidence of serious depletion according to authority cited above. The present investigators prefer to interpret this low fasting excretion of riboflavin as evidence of low current consumption of the vitamin rather than sure evidence of desaturation of body stores. Ten subjects excreted so little riboflavin (less than 2 micrograms) per hour that the methods used were incapable of measuring the concentration. Only 12 per cent of the group excreted more than 20 micrograms of riboflavin per fasting hour.

The average excretion of 11.2 micrograms in the present group is in sharp contrast with the average of 26 micrograms of urinary riboflavin per fasting hour reported in the Colorado tests on normal American troops in the States. The lower excretion of riboflavin in the Manila troops is consistent with their lower performance in fasting thiamine excretion compared with the records of the Colorado test subjects. This behavior suggests a lower plane of riboflavin nutrition in the Manila troops than in the representative normal troops in the United States.

11. Vitamin B_2 Excretion in Urine After Oral Test Dose (5 Milligrams of Riboflavin).

Load test responses to riboflavin were measured in 98 of the subjects. The principles involved in administration of the "load" test dose of riboflavin and interpretation of response are similar to those described for the thiamine "load" test, above. According to the Armored Medical Research Laboratory and Harvard Fatigue Laboratory Reports (cited above), 200 micrograms

or urinary riboflavin per 4 hours subsequent to the oral test dose is the minimal "normal" response. Any excretion below 200 micrograms of riboflavin is presumptive evidence of desaturation in severe degree. The upper range of normal response is stated as 2500 micrograms per 4 hours.

The experimental group, during the Vitamin B_2 load tests, showed extreme responses of 0 to 2380 micrograms of urinary riboflavin in 4 hours. The average urinary excretion in the test was 829 micrograms of riboflavin per 4 hours and the median and mode appeared also in the range of 800 to 900 micrograms. Table 13 and Chart 4 (Inclosure 6) contain full details of the riboflavin load test data.

Only 6 of the 98 subjects excreted less than 200 micrograms of urinary riboflavin in the 4-hour load test and are classed as severely depleted with respect to Vitamin B_2, according to current interpretation. These load test data are in sharp contrast to the results of the fasting test which gave evidence of severe depletion in over 60 per cent of the subjects. The cause of this discrepancy is not known but the present investigators favor the interpretation of nutritional status based on load test responses. No one knows the effect of tropical environment on the metabolism of the B-vitamin complex and tropical conditions might be operative in producing the present discrepancy between fasting and load test responses. It is evident, in the case of Vitamin B_2 as in Vitamin B_1 tests, that fasting urinary excretion and response to load tests are not closely related and appear to measure different factors in the metabolism of the vitamin.

The average response in riboflavin load tests in the present (Manila) group of 829 micrograms, is in contrast to the average response by representative group of American troops in the Colorado Tests (cited above), who excreted about 1600 to 1700 micrograms per 4 hours. Only 6 of the 98 subjects

in the present study excreted more than 1600 micrograms per 4 hours, which suggests that the present group is in a lower plane of nutrition with respect to riboflavin than were the "stateside" troops in the Colorado tests.

There was general failure of correlation between fasting urinary riboflavin excretion data and load test responses. Even where extreme ranges only are considered the correlation is unimpressive. For example: Six subjects giving the highest responses to the load test (average 1860 micrograms of riboflavin) had an average fasting hourly excretion of urinary riboflavin of 13.6 micrograms and three of these six subjects had fasting excretions of less than 10 micrograms (abnormally low fasting excretion). In contrast to this, 6 subjects who had the lowest load test responses (average 110 micrograms of riboflavin) gave an average fasting hourly excretion of 8.8 micrograms and two of these subjects had excretions above 10 micrograms per fasting hour (adequate fasting excretion).

12. Correlation Among the Series of Observations.

Inasmuch as observations on the various vitamins had been made on the same set of subjects it was apparent that the testing of correlation among the nutritional findings would evolve the most pertinent interpretations. Correlations were tested by preparing scatter diagrams, plotting each set of observations against each of the others. The scatter diagrams revealed that close correlations could not be obtained over the whole range of subjects and that further statistical treatment of the whole group would produce little of value in an understanding of factors involved in the nutritional state.

Such consistent failure of correlation of the variable factors, however, should be anticipated where the principal data are secured within the normal range of nutrition. It seems logical that within the normal range, where

nutritional matters do not act as limiting factors, other complicated forces might produce variations which in turn would mark relationships which the study was designed to test. Reasoning from this point of view, it appeared that the use of only extreme data would reveal correlations more clearly. Consequently only the subjects showing highest and lowest performance in each test were used in correlation studies and the subjects accupying the middle ranges were omitted. Such a treatment of data has been rewarded with evidence of correlation among the biochemical data and physical findings in the subjects. Correlations, that seem most pertinent to understanding the nutritional state of the Manila group, are discussed in the inclosures which follow.

Inclosure 6

Results and Interpretations

Part II

Study of Selected Subjects Showing Either High Degree of Saturation or Partial Depletion in Thiamine and Riboflavin.

General Comment.

The chemical tests have revealed the physiological behavior of a representative group of soldiers in the Manila Area in the storage and excretion of certain vitamins and minerals and as regards the state of their blood proteins. There has been obtained, from these studies, evidence of general adequacy of nutrition in this area but also a tendency to lower plane of nutrition than in normal troops in the United States. Some of the subjects in the present study have given chemical evidence of being in a state of partial depletion in one or more vitamins although none exhibited a state of evident illness.

The range of performance of subjects in the bio-chemical tests was wide. The principle interest in the study centers around those subjects who made the poorest records and a comparison of these subjects, presumed to be in a state of partial depletion, with the subjects who made the best records and who are undoubtedly well saturated with the vitamins under consideration.

Specific interest centers about the B-vitamins because of the profound effect these have upon metabolism and body functions. Experience in the construction of troop dietaries, quality of rations, and troop feeding in the Pacific area has led the present workers to suspect that where lowered nutritional state has ever operated to the detriment of health or efficiency of troops it has probably done so through deficiency of the Vitamin B-complex.

With this interest in B-complex, the present Inclosure has been prepared as a comparison of subjects who showed partial depletion with those who gave evidence of a good degree of saturation in thiamine and riboflavin. For purposes of the comparison arbitrary selection of subjects has been made on the basis of performance in load tests which are believed to measure, most accurately, the state of vitamin saturation of the body. State of saturation with respect to riboflavin alone, thiamine alone and riboflavin and thiamine together have formed the basis for selection. Six categories of subjects have been chosen and each category contains about twenty subjects who occupy the extreme ends of the range of behavior in load tests. Comparisons are made of the "extreme" subjects with the omission of about 60 or 70 subjects occupying the middle of the performance range and who, because of their apparent normalcy, tend to obscure correlations among the data.

The six categories of subjects are those showing:

(a) Highest response to thiamine load test.

(b) Highest response to riboflavin load test.

(c) Highest response to both thiamine and riboflavin load tests.

(d) Lowest response to thiamine load test.

(e) Lowest response to riboflavin load test.

(f) Lowest responses with respect to both riboflavin and thiamine load tests.

There is considerable overlapping of subjects in the three low categories with each other and in the three high categories with each other inasmuch as body saturation with respect to thiamine and riboflavin tend to run generally parallel. The limits of performance in load tests of the arbitrarily selebted groups of subjects are presented in Table 14, Inclosure 8. Thus the subjects in low performance categories show levels in the load tests usually at or only slightly above

those considered to demonstrate severe depletion, while subjects in the high performance categories show levels in load tests which compare favorably or are above those found in the troops in the United States.

For ease in terminology in the discussions which follow, the term "low-score subjects" will be used to refer to those subjects who made low excretions during load testing. These will be compared with the "high-score subjects". This terminology eliminates the use of ill defined terms as "deficient subject". The term "deficiency" in vitamin nutrition has not yet been clearly enough defined and at the present time can be applied with impurity only to a subject having suffered severe depletion of sufficient duration and degree to produce obvious and specific clinical symptoms. Such concept of "deficiency", while it must stand unviolated at the moment, is inevitably doomed to revision as soon as bio-chemical methods evolve to the point of fully revealing the more insidious "sub-clinical deficiency" state.

2. Observations which Fail to Correlate with B-Vitamins.

Many of the observations made in connection with this study failed to show any correlation with state of thiamine or riboflavin nutrition. Such failure of correlation was demonstrated by showing that "low-score" groups do not differ in certain characteristics from "high-score" groups. The following failed to correlate with the B-vitamins:

(a) Height, weight, or weight-height ratio.

(b) Age

(c) Months of overseas service.

(d) Plasma protein concentration.

(e) Sodium chloride excretion in urine during fasting.

(f) Ascorbic acid (Vitamin C) excretion in urine during fasting.

(e) Ascorbic acid concentration in blood.

a. Height and weight.

No evidence exists in the present group of a relationship between height or weight or their ratio and nutritional state with respect to B-Vitamins. Thus, there were as many slender subjects in the low-score groups as in the high-score groups and weight-height ratios indicating relative stoutness in body form were as prevalent in the low as in the high-score groups. This conclusion does not rule out the possiblity that subjects showing more severe depletion than the present group might reveal some relation of Vitamin B_1 and Vitamin B_2 nutrition with body cohtour.

b. Age in years was not an apparent factor differentiating high- and low-score groups.

c. Overseas Service.

Months of tropical overseas service bore no relationship to state of Vitamin B-complex saturation. No greater proportion of men, who had long overseas service, attained low scores in the load testing then attained high scores. Thus, time of exposure to the overseas and tropical conditions appeared to have no bearing on B-vitamin nutrition even though the experimental group as a whole showed a lower plane of nutrition than did subjects in the Colorado Tests on representative "stateside" troops.

d. Plasma protein.

Plasma protein concentrations were of similar distribution in high- and low-score groups, demonstrating no relationship of plasma protein levels with performance in the thiamine and riboflavin load test. Reference is made to Table 6 (Inclosure 8) in which the distribution of plasma protein is fully presented for the whole series of subjects as well as

for groups in each of the high- and low-score categories.

e. Fasting urinary chloride.

The excretion of sodium chloride during the fasting demonstrated that about 25 per cent of the subjects might be low in this electrolyte. However, there was no more evidence of salt desaturation in the low-score groups than in the high categories. Reference is made to Table 5 (Inclosure 8) for a comparison of salt excretions per fasting hour with attainment on thiamine and riboflavin load tests.

f. Vitamin C in fasting urine and blood.

Nutritional state, with respect to Vitamin C as determined either by fasting urinary excretion or concentration in blood, bore no apparent relation to nutritional plane with respect to thiamine and riboflavin. Tables 8 & 9 (Inclosure 8) show the distribution of blood and fasting urinary levels of Vitamin C in the high- and low- thiamine- and riboflavin-score groups.

Failure of the plane of Vitamin C nutrition to relate to plane of Vitamin B nutrition in the present group is consitent with known facts concerning the composition of foods. Foods such as meat, whole grain cereals, eggs and milk are good dietary sources of thiamine and riboflavin and other B-complex components but they are poor sources of Vitamin C. On the other hand fruit, fruit juices and vegetables are good sources of Vitamin C but generally of little importance (in the usual quantities consumed) in furnishing the B-vitamins. Thus, it is entirely possible to choose a dietary furnishing either ample vitamin B-complex or Vitamin C and containing but little of the other. Where appetite fails, due to any cause, people frequently tend to consume fruit and fruit juices when these items appear in the menu and will exclude high caloric foods rich

in the Vitamin B-complex. In contrast to this, there are many who, from life-long habits, choose dietaries almost exclusively of bread, butter, meat and potatoes. Such individuals would have excellent nutritional status with respect to B-vitamins and be somewhat desaturated with respect to Vitamin C.

3. The Relationship of Vitamins B_1 and B_2 to Blood Hemoglobin.

The present study, within the limits imposed, presents evidence that state of saturation of the subject with thiamine and riboflavin bears some relationship to blood level of hemoglobin. This relationship became apparent when high- and low-score subjects (determined in load testing) were compared. While the study does give evidence of a relationship, it cannot determine whether thiamine or riboflavin desaturation or both are directly involved; nor whether the relationship obtains indirectly through one or more other factors. Thus, lowering of blood hemoglobin might be due to partial desaturation in pyridoxine or nicotinamide or other nutritional factors of the B-complex frequently related to low Vitamin B_1 and B_2 scores. In other words, the study <u>can not</u> differentiate the role of thiamine and riboflavin as principle factors, or as accompanying and indirect factors.

Reference is made to Table 7 and Chart 3 (Inclosure 8) for a full presentation of the blood hemoglobin concentration ranges among the high- and low- B-Vitamin-score groups. The general tendency is for the high-score groups to be associated with higher hemoglobin levels and low-score groups to be associated with lower hemoglobin levels.

The twenty subjects showing low-riboflavin scores in the load test (average urinary riboflavin of 266 micrograms per 4 hours) had an average hemoglobin concentration of 15.3 grams per 100 cc of blood. In contrast twenty-one subjects with high-riboflavin scores (average 1466 micrograms per 4 hours) had, on the

average, 16.9 grams of hemoglobin per 100 cc of blood. The whole experimental group (108 subjects) had an average hemoglobin concentration of 16.0 grams per cent, midway between the hemoglobin levels of the high- and low- riboflavin-score groups.

The low-riboflavin-score group (20 subjects) contained three subjects who had less than the normal hemoglobin concentration (15 grams per 100 cc of blood) and thirteen subjects who had less than 16 grams per cent hemoglobin. The hemoglobin maximum in this group was 18.5 grams per cent and the minimum, 13.8 grams per cent.

In contrast, the high-riboflavin-score group (21 subjects) had none showing the minimal normal hemoglobin level of 15 grams per 100 cc of blood. Only three subjects had less than 16 grams per cent hemoglobin. The hemoglobin maximum in the high-score group was 20.1 grams per cent and the minimum 15.1 grams per cent.

A very striking discrepancy in hemoglobin concentration is evident when the group making high scores in both thiamine and riboflavin load tests are compared with the corresponding low-score group. The discrepancy in distribution of values is in the same direction found by the comparison of high- and low-riboflavin-score groups; the same relationships is also evident in the comparison of high- and low-thiamine-score groups, though somewhat less conspicuous.

It is of particular interest that, even though the hemoglobin concentrations in all 108 experimental subjects did not extend far beyond the so-called "normal" range, there was a tendency for high- and low-B-vitamin-score groups to show differences in the hemoglobin levels. The bio-chemical literature contains many reports of the B-complex vitamin deficiencies producing anemia. Anemias

in experimental animals, due to thiamine and to pyridoxine deficiencies, are frequently described.

4. Relationship of Vitamin B_1 with Vitamin B_2 Load Test Scores.

The study presents evidence of a correlation between responses to load tests of thiamine and riboflavin. This correlation, which is consistent to a fair degree over the whole range of experimental subjects, becomes most apparent when the high- and low-score subjects are compared. The tendency is for high response in thiamine load test to be associated with high response to riboflavin load tests and for low riboflavin scores to appear in those subjects having low thiamine scores. There are only a few exceptions in which low-score with respect to one of B-vitamins occurs in a subject having an average score in the other. Reference is made to Table 15 (Inclosure 8) which presents the average response scores in riboflavin and thiamine load tests in high- and low-score groups.

The high-riboflavin-score group (average score was 1466 micrograms of riboflavin) had an average urinary thiamine excretion during the 4-hour load test of 180 micrograms, which is above the average (150 micrograms) for the whole group of subjects. The low-riboflavin-score group (average score was 266 micrograms of riboflavin) had an average urinary thiamine excretion during the 4-hour load test of 89 micrograms.

Likewise the high-thiamine-score group (average score was 278 micrograms of thiamine) had an average urinary riboflavin excretion of 1016 micrograms which is above the average (829 micrograms) for the whole group of subjects. The low-thiamine-score group (average score was 46 micrograms of thiamine) had an average urinary riboflavin excretion during the 4-hour load tests of 456 micrograms.

Further evidence of direct correlation among thiamine and riboflavin scores is shown by the fact that in 20 subjects classed in the low-riboflavin-score group, 10 of these subjects appeared in the low-thiamine-score group.

Correlation of planes of nutrition as related to thiamine and riboflavin is consistent with findings in clinical work with malnutrition, in which frank deficiency of only one member of the Vitamin B-complex is seldom found. Such deficiencies are generally multiple.

The relationship of thiamine with riboflavin has a logical explanation in that:

a. Food sources rich in riboflavin are generally rich in thiamine and poor dietary sources of thiamine are poor sources of riboflavin. Undoubtedly nutritive quality of food consumed plays a part in correlating these two members of the Vitamin B-complex.

b. Thiamine and riboflavin have similar roles in body physiology, both acting as carrier-catalysts in the enzyme systems regulating cellular respiration. Therefore, whether dietary quality or state of metabolism determines the level of body stores of thiamine, it will exert an influence on the level of body stores of riboflavin in the same degree and direction.

5. Appetite, Morale and Personal Medical History Related to Vitamin B_1 and Vitamin B_2 Planes of Nutrition.

Evaluation of morale and appetite in the subjects presented difficulties inasmuch as these are subjective matters. The questionaire method was used, in which each subject evaluated his own subjective feelings as to morale and appetite. The use of the confidential questionaire in this connection has already been described in Inclosure 5. At the same time these questionaires were prepared by the subjects, a second questionaire on personal medical his-

tory was also completed. Appetite and morale were each graded simply as poor, fair, good ar excellent. The medical history related to diseases and disorders which had occurred during the 6 months prior to experiment.

In the medical histories described herein, the subjects were given entire freedom of reporting. The results of the medical history, therefore, are in fact more or less distorted by any tendency to hypochondria existing in each subject. In Inclosure 7, which follows, medical histories are reported which were obtained by experienced clinicians, and are distinct from the ones considered in this section.

a. Appetite

Subjective reaction reported in the appetite questionaire is not regarded by the present investigators as indicative purely of state of appetite. It may be, in individual cases, a reflection of other attitudes manifesting themselves in evaluation of attitude toward food.

Table 17 & Chart 1 present the findings in the appetite questionaire in all of the subjects and the distribution of appetite ratings in groups attaining high and low scores during the Vitamin B_1 and B_2 load tests. The general tendency is for proportionately more fair and poor appetite ratings to appear in low-score groups and more good appetite ratings to appear in the high-score groups.

For example, the group of 20 high-thiamine and riboflavin-score subjects reported 13 good, 5 fair and 2 poor appetites. The group of 20 subjects having low scores in both riboflavin and thiamine reported 7 good, 9 fair and 4 poor appetites. A similar trend appears when groups having high and low scores with respect to either riboflavin or thiamine alone are compared.

The data suggest that complaints of somewhat poorer appetite may be expected in subjects making lower scores in thiamine or riboflavin load tests. The results are only suggestive and do not assist in differentiating poor appetite as cause or effect of Vitamin B desaturation. Furthermore, the results do not indicate whether the relationship, is direct or indirect (through other related factors).

b. Morale

Morale ratings present difficulties similar to those found in interpretation of appetite ratings when related to plane of thiamine or riboflavin nutrition. The general tendency is observed for better morale ratings to appear more frequently in high load test score groups and for poorer morale ratings to appear more frequently in low load test score groups. The morale ratings in the whole experimental group and in high and low load test score groups are given in Table 16 and Chart 2 (Inclosure 8).

Of 20 subjects having high scores in both Vitamin B_1 and Vitamin B_2 load tests, 2 reported excellent morale, 13 good, 5 fair and none poor. In contrast in the group of 20 subjects attaining poor scores in both Vitamin B_1 and Vitamin B_2 load tests, none reported excellent morale, 8 good, 8 fair and 4 poor.

A similar relationship appears when low-thiamine- and high-thiamine-score groups or low-riboflavin- and high-riboflavin-score groups are compared.

c. Personal medical histories.

Personal medical histories were of special interest in relation to state of thiamine and riboflavin nutrition. In general there was greater tendency for complaint of diseases within the past 6 months in the low-

score groups than in the high-score groups. Whether the diseases reported were real or imagined is not known, but the higher incidence of complaints in subjects, judged to be severely or partially desaturated in thiamine or riboflavin or both, is worthy of emphasis.

The occurence of reported disorders during the past 6 months, as given in the subjects self-written medical histories, are presented fully in Table 18 (Inclosure 8), showing the records of high- and low-riboflavin- and thiamine-score groups.

Twenty subjects showing highest scores in both thiamine and riboflavin reported no disorders in 15 subjects and the other 5 subjects reported 3 skin disorders (unclassified) and 2 acute diarrheas. In contrast the 20 showing poor scores in both thiamine and riboflavin load tests reported 11 with no disorders, 6 with skin disorders, 6 cases of acute diarrhea, 2 malaria and 2 dengue fevers. Some of the disorders in the low-score group were multiple, two or more occurring in the same individual.

Inasmuch as interpretation of medical history is properly a clinical problem, the principal discussion will be left for the section, on Clinical Observations, which follows (Inclosure 7). Let it suffice here to say, only, that complaint of greater numbers of disorders like poorer state of morale and poorer appetites appear to concentrate in the groups attaining lowest scores with respect to thiamine and riboflavin.

d. General Comment.

The findings suggest that sense of well-being is not as great in the groups showing a lowered plane of nutrition. The correlation, of nutritional state with subjective feelings and general health has long been suspected but never proven. The present work serves to emphasize the probable correlation.

Inclosure 7

Clinical Observations

1. General Comments.

Three weeks after the completion of all biochemical observations, physical examinations were carried out by clinicians on a series of subjects selected from the original group on the basis of performance in chemical tests. The physical examinations therefore post dated the chemical observations by three to nine weeks. The purpose of the examinations was two-fold:

a. To determine existence of any specific clinical signs of early avitaminosis in those subjects, who, according to chemical tests, could be classified as severely desaturated or closely approaching desaturation in thiamine and riboflavin.

b. To determine whether subjects making poor records in chemical tests showed any signs of lower state of general health or higher incidence of disease than did subjects saturated in good degree with these Vitamin B-complex components.

The investigators are grateful to three medical officers who generously contributed their time and experience in examining the selected series of subjects. Major Bertram Nelson, MC and Captain Richard Morris, MC, of the 13th General Hospital made the physical examinations and Major Frank Greene, MC, ophthalmologist of the 60th General Hospital carried out slit lamp examination for proliferation of corneal capillary vessels (an important clinical test for early riboflavin deficiency). The 60th General Hospital, San Beda College, provided space and equipment for use in the examinations.

At the time of physical examination photographs were taken of each subject wearing shorts only, to secure a permanent record of general appearance

of the subjects and their body dimensions. Photographing was done by the 8th Medical Arts Detachment and reproductions of the pictures will be on file in the army Medical Museum and obtainable through the Office of the Surgeon General, War Department, Washington D. C., by reference to File numbers H0031 through H0064 inclusive.

Subjects for physical examination were selected from the original experimental group of 111 soldiers on the basis of performance in load tests with Vitamin B_1 and Vitamin B_2. Sixteen subjects, who constituted the series referred to in the following discussion as the "high-score" group made high load test records with thiamine and riboflavin. Their performance compared favorably with the average performance observed in the Colorado tests on representative troops in the States (referred to in Inclosure 5 and 6).

Twenty-seven subjects who constituted the series referred to in the following discussion as the "low-score" group, made low records in load test with either thiamine or riboflavin or both. Twenty of the "low score" subjects made low or severely low records in both thiamine and riboflavin. The remaining seven made very low records only in the thiamine or the riboflavin test alone and showed less than average performance in the other. Thus 20 subjects in the low-score group could be classified as in a state of multiple desaturation as judged by chemical tests while the other 7 subjects showed desaturation in only one of the two B-vitamins studied.

At the time of physical examination most of the subjects originally tested by biochemical methods were still available for clinical study. The limits of performance of high- and low-score groups referred to in Inclosure 6 and described in Tables 14 and 15 apply approximately for high- and low-score subjects used in the clinical observations.

The clinicians felt that impartial examination of subjects could be best secured if the examining doctors had no prior knowledge concerning load test performance of each subjects. Therefore, "high and low score" subjects were brought to physical examinations in random order. Only after completion of all the clinical observations, were the biochemical data revealed to the clinicians or to the subjects. All of the interpretations of clinical data were made only after several conference with the examining physicians in which the findings were fully discussed. One special concern of the physicians was the fact that a substantial time had elapsed between biochemical testing and physical examination and it was their opinion that subtle clinical signs of avitaminosis may appear and disappear rapidly with changes in nutritional state. It is the opinion of the present investigators that the dietary in the Manila area would do little to lower or raise degree of saturation in subjects over the period which elapsed between biochemical tests and clinical observations. However, the general failure to find physical signs of specific avitaminosis at the time of clinical examination does not entirely rule out the possibility that such signs might have existed in some low score subjects at the time of biochemical testing.

2. Medical Histories.

The medical histories obtained during physical examination cover only the 6 months prior to study (March through August, 1945). The subjects were questioned on the several pertinent matters which follow. Reference is also made to the summary of Medical Histories in Tables 19 and 20 (Inclosure 8).

a. Number of Hospitalizations.

The 16 "high-score" subjects reported only 1 hospitalization over the 6 months period prior to study. The 27 "low-score" subjects reported 9

hospitalizations, a substantially higher rate.

b. Sick calls to Unit Dispensaries.

Sixteen high-score subjects reported a total of 26 sick calls for all causes, with 7 subjects having made no sick calls during 6 months. The 9 subjects who made any sick calls reported a frequency of 1 to 6 visits to the dispensary. In the high-score group, therefore, there were 1.6 sick calls per subject and only 56 per cent of the group had reported on sick call one or more times. The low-score group show proportionately a much greater incidence and frequency of sick call. Twenty-seven low-score subjects reported a total of 119 sick calls, with 5 subjects having made no sick calls. The 22 subjects who made any sick calls reported a frequency of 1 to 15 visits to the dispensary. In the low-score group, therefore, there were 4.4 sick calls per subject and 85 per cent of the group had reported on sick call one or more times.

c. Acute respiratory infections.

A comparison of reports on the number of acute respiratory infections demonstrates that frequency of these disorders was definitely greater in the low- than in the high-score groups.

Twenty-six low-score subjects reported a total of 53 acute respiratory infections during the 6 months prior to examination. The twenty-seventh subject reported infections of such frequency that he was classified as a chronic case and not included in the total. Thus the average incidence for the group was 2.0 attacks per man with a frequency of 1 to 10 attacks in each subject reporting the disorder. Only 6 of the 27 men (23 per cent) reported no chronic or acute respiratory infection.

Sixteen high-score soldiers reported 18 acute respiratory infections

during the same period and no chronic cases were observed. Thus, the average incidence was 1.1 attacks per man with a frequency of 1 to 6 attacks in each subject reporting the disorder. Nine of the 16 men (56 per cent) reported no acute respiratory infection.

d. Acute diarrhea.

The data on acute diarrheas was more striking than that on respiratory infections in demonstrating the difference between high-score and low-score subjects. Frequency of acute and chronic diarrheas in the low-score group was remarkably raised.

Twenty-five low score subjects reported a total of 55 attacks of acute diarrhea during the 6 months prior to examination. The other two subjects reported two to four watery stools daily and were classed as having chronic diarrhea. Thus, in twenty-five subjects, the incidence per man was 2.2 episodes for the period under consideration with a frequency of 1 to 10 attacks in those reporting acute diarrhea. Only 7 of the 27 subjects (26 per cent) reported no acute or chronic diarrhea.

The 16 high-score subjects reported a total of only 3 acute diarrheas or 0.2 attacks per man. The attacks occurred in only two subjects and 14 had suffered no diarrheas (88 per cent of the group). There were no chronic diarrheas diagnosed in the high-score group.

e. Insomnia, Headaches and Feelings of Faintness on Arising.

Sixteen subjects in the high-score group reported no insomnia, no chronic headaches and no feeling of faintness on arising. In contrast, among the 27 low-score subjects there were 4 cases reporting insomnia (15 per cent) 7 cases reporting chronic headache (26 per cent incidence, 5 subjects had the symptom frequently and 2 had the symptom occasionally), and 6 cases

of faintness on arising (22 per cent incidence).

It is not known whether these three symptoms existed in fact or whether the high incidence might have been the result of desire to complain of ill health.

f. General Endurance (Subjective Evaluation).

When questioned concerning physical endurance, 9 of the 27 low-score subjects (33 per cent) complained of noticeable lowering of endurance while 3 of 16 high-score subjects (20 per cent) complained of the same subjective feeling. The difference between the low- and high-score groups in evaluation of general endurance does not appear significant nor does it indicate that the low-score group had a lower sense of well-being.

g. Summary of Medical History.

Frequency of hospitalization, sick call to unit dispensaries, episodes of acute respiratory infections and diarrhea, and incidence of insomnia, chronic headache and feeling of faintness on arising were all greater in the low-score than in the high-score groups. These differences between high-and low-score groups appear to be significant and characteristic of the subjects studied in the present experiments.

One cannot rule out, even in a well-controlled medical histories, the possibility of generally lowered sense of well-being and desire to complain of ill health coloring the results in the low-score group. Frequent visits to unit dispensaries may manifest to some extent feelings of insecurity or apprehension rather than true diseases of more than minor character.

The relation between acute infection and plane of B-Vitamin nutrition can be interpreted in a variety of ways. Among the possible interpretations are:

(1) Partial depletion of B-complex vitamins may be the result of acute and chronic disorders.

(2) Partial depletion of B-complex vitamins may predispose the subject to acute disease or act as the causative factor.

(3) The correlation between nutritional plane in B-complex Vitamins and incidence of disease might be coincidental or might result through other causative factors common to both.

The present survey does not give an indication as to the correct interpretation; but proves only that the degree of desaturation with respect to B-vitamins observed in the low-score group was coexistant with acute and chronic disorders which are known to reduce efficiency in full duty troops.

3. Nervous System Signs and Symptoms.

a. Paraesthesias.

Paraesthesias were recorded only in two instances. Both appeared in the low-score group of 27 subjects. None were reported in the high-score group.

b. Nerve Tenderness.

Nerve tenderness was determined by pressure over the peroneal nerve at the head of fibula with force adjudged not to cause pain in a normal subject.

Nerve tenderness was observed in marked degree in one subject and in a mild degree in two others. All three of the cases of nerve tenderness appeared among the low-score group (27 subjects), no cases being found in

the high-score group (16 subjects).

c. Leg tendon reflexes.

Three instances of leg tendon reflex abnormalities were observed in the low-score group (27 subjects). Complete absence of pattellar and tendon Achilles reflexes was observed in one of these subjects, another had the patellar reflex but no tendon Achilles reflex in either leg, and in the third subject absence of the patellar and tendon Achilles reflex was unilateral only. No leg tendon reflex abnormality was found among the 16 high-score subjects.

4. Ocular Signs.

a. Slit lamp examination.

Examination of the cornea of the eye was made by the participating opthalmologist to secure evidence of vascular proliferation in the high- and low-score groups. No signs of increased vascularity were observed which could be classed as pathological. Six cases of mild bilateral "twig" capillary formation from the limbi were noted but no instance of complete capillary looping (characteristic of ariboflavinosis) was found. Four of the six cases of mild "twig" formation (interpreted as consistent with normalcy) appeared in the low-score group (27 subjects) while two appeared in the high-score group (16 subjects). It is concluded that none of the subjects in the present study were sufficiently desaturated with respect to riboflavin to show increased corneal vascularity, the first observable clinical sign of early ariboflavinosis.

b. Conjuctivitis.

Examinations also indicated some mild to moderate conjunctivitis in the subjects but frequency of occurence did not reveal a great difference between high- and low-score groups. Ten of the 27 low-score subjects

(38 per cent) showed this symptom as did 4 of the 16 high-score subjects (25 per cent).

c. Other eye symptoms.

Three of the 27 low-score subjects complained of eye discomfort. One reported phoyophobia, another reported photophobia and burning and the third burning and lachrimation.

Only one of the 16 high-score group complained of eye discomfort, reporting excessive lachrimation only.

6. Oral Signs.

Gingivitis was diagnosed in 4 of the 27 low-score subjects and 2 of the 16 high-score subjects, showing no tendency in the present work to relate to plane of Vitamin B_1 or B_2 nutrition. It is also of interest to note that low blood Vitamin C levels were not correlated with incidence gingivitis.

Aphthous ulcers (cancre sores) were complained of in one subject of the high-score group (16 subjects) and in 4 of the low-score group (27 subjects).

Glossitis has been described by many workers as a frequent accompaniment of deficiencies in the Vitamin B complex and more especially associated with inadequate riboflavin and nicotinic acid intakes. The physicians examined subjects in the present study for glossitis but did not consider that the observation of glossitis in extremely mild degree could be interpreted clinically. According to their diagnosis, the very mild changes observed in the subjects were "minimal redness accompanied by mild hypertrophy of the fungiform papilli and atrophy of the filliform papilli at the tip with or without similar changes at the edge of the tongue but not referrable to dental prosthetics". Such mild changes appeared in 11 of the 27 low-score subjects (41 per cent) and in 4 of the 16 high-score subjects (25 per cent). The distribution among groups does

not seem to present any striking evidence of difference and suggests that this very minimal type of change in the tongue surface is not of the type indicating riboflavin or thiamine deficiency.

Cheilosis is a frequent accompaniment of ariboflavinosis. The clinicians found no cheilosis of a degree considered significant in any of the subjects. They listed minimal or doubtful cheilosis in 6 of the 27 low-score subjects (22 per cent) and in 2 of the 16 high-score subjects (12 per cent).

7. Epidermal Signs and Symptoms.

a. Acne, Folliculitis and Impetigo.

Classification of skin disorders observed in 27 low-score subjects resulted in the following: 4 acne (moderate), 2 impetigo, 4 folliculitis and 1 case of seborrheic dermatitis. This made a total of 11 cases but inasmuch as multiple infections occurred wihhin the group only 9 of the 27 subjects were involved (33 per cent).

In 16 high-score subjects, there appeared 1 acne (mild), 1 impetigo, 1 folliculitis and 1 seborrheic dermatitis. Thus 4 cases occurred in the group (25 per cent).

The clinical interpretation is that no significant differences exist between high- and low-score groups in relation to these "non-specific" skin disorders.

b. There were no cases of dermatitis characteristic of pellagra in any subjects.

c. Facial dermatitis of the type frequently associated with ariboflavinosis was not observed in any of the subjects. Scaliness and redness (not believed of fungus origin) was observed in pubic region and on the

scrotum in 14 subjects but distribution of these subjects within high and low score groups was so even that no significant difference appeared.

d. The only skin sign considered of possible significance in regard to the selected groups was a peculiar lesion located in the sacro-coccygeal area between the buttocks. The clinicians have described this as follows: "a poorly demarked lesion in the sacro-coccygeal area between the buttocks with slight maceration and tendency to fissure and with no obvious sign of ringworm as the causative agent." The lesion was found in 12 of the 27 low-score subjects (44 per cent) and in very mild degree in only one of the 16 low-score subjects (6 per cent). This sign alone, of all the skin observations was believed of possible significance in differentiating the high- and low-score groups.

8. Liver Enlargement.

Examination revealed one subject in the high-score group (6 per cent) having a palpable liver extending 1 finger width below the costal margin. There were 6 having palpable livers extending 1 to 2 finger widths below the costal margin in the low-score group (22 per cent) and one subject showing liver tenderness with no enlargement. It is believed that the difference in distribution of palpable livers in high- and low-score groups may be of some significance in relation to nutritional state.

9. Physical Fitness.

There was no difference observed in physical fitness between low- and high-score groups. Fitness scores determined throught the use of a simple stepping test followed by observation of pulse and blood pressure. There was no difference between the groups in their response of systolic blood pressure to the postural change, from lying to standing position.

10. Summary and Interpretation of Clinical Signs.

a. The medical histories revealed a higher occurence in low- than in high-score subjects of hospitalizations and sick calls to the unit dispensaries. This differance is consistent with the higher incidence of acute respiratory and diarrhea in the low-score group as well as more frequent complaints of headache, insomnia and feeling of faintness on arising.

b. There were no clinical findings in any subjects which could be clearly related to avitaminosis. However, a peculiar skin lesion located in the sacro-coccygeal area between the buttocks was found frequently in low-score subjects and is believed of possible significance in relation to nutritional condition.

c. The cases of paresthesia, leg tendon reflex abnormalities and nerve tenderness were not frequent but all appeared in low-score groups.

d. Ocular, oral and epidermal symptoms which did occur were all of mild or very mild character. These tended to appear somewhat more frequently in the low-score than in the high-score group but the difference was neither marked nor of such severity that the symptoms could be interpreted as indicative of lower nutritional state.

e. Examination of viscera revealed more palpable livers in the low-score group than in the high-score group.

INCLOSURE 8

TABLES AND CHARTS

TABLE 1

DISTRIBUTION OF AGES OF SUBJECTS

Age in Years	Per cent of subjects
18-20	13.5
21-23	23.4
24-26	26.1
27-29	15.3
30-32	7.2
33-35	6.3
36-38	6.3
39-41	1.9

Average age--25.6 years

TABLE 2

DISTRIBUTION OF HEIGHTS AND WEIGHTS OF SUBJECTS

Weight (in pounds)

ight in ches)	100 109	110 119	120 129	130 139	140 149	150 159	160 169	170 179	180 189	190 199	200 209	210 219	220 229	230 239	240 249	TOTAL
-61 3/4					1											1
-62 3/4																0
-63 3/4	1															1
-64 3/4	1			1	1											3
-65 3/4				2		1	2									5
-66 3/4				1	3	3		1								8
-67 3/4		1			4	2	2									9
-68 3/4				1	5	6	2	2								16
-69 3/4					2	5	3									10
-70 3/4					4	6	4		3	1	2				1	21
-71 3/4					2	2	5	3	4		1		1			18
-72 3/4					1	1	5			1						8
-73 3/4				1			1		2		1					5
-74 3/4						1		1	1					1		4
-75 3/4							1	1								2
TAL	2	1	0	6	23	27	25	8	10	2	4	0	1	1	1	

Average weight -- 159 pounds

Average height -- 70 inches

TABLE 3

DISTRIBUTION OF WEIGHT/HEIGHT RATIOS OF SUBJECTS

Weight (pounds) divided by height (inches)	Per cent of subjects
1.6-1.7	2.7
1.8-1.9	0.9
2.0-2.1	23.4
2.2-2.3	38.8
2.4-2.5	18.0
2.6-2.7	9.9
2.8-2.9	3.6
3.0-3.1	1.8
3.2-3.3	----
3.4-3.5	0.9

Average ratio -- 2.3

TABLE 4

NORMAL RANGES AND LEVELS* SUGGESTIVE OF DEFICIENCY SERIOUS ENOUGH TO IMPAIR MORALE, PHYSICAL FITNESS, AND GENERAL HEALTH IF LONG ENOUGH CONTINUED.

	Normal range	Level suggestive of serious deficiency
Whole blood hemoglobin (Gms/100 cc)	15 to 19	Below 12.0
Serum protein (Gms/100 cc)	5.8 to 7.0	" 5.2
Urinary chloride (Gms NaCl 3/4 hr)	0.2 to 1.0	" 0.2
Fasting urinary Vitamin C (Mgm/hr)	0.3 to 1.0	" 0.3
Fasting urinary Vitamin B_1 (Mcgm/hr)	2 to 25	" 2
Load test Vitamin B_1 (Mcgm/4hr)	50 to 800	" 50
Fasting urinary Vitamin B_2 (Mcgm/hr)	10 to 100	" 10
Load test Vitamin B_2 (Mcgm/4hr)	200 to 2500	" 200

*Extracted from Table 1, Section VII (Biochemical Studies, Summary of Results) of the final report (22 November 1944) on Project No. 30, "Test of Acceptability and Adequacy of U. S. Army C, K, 10-in-1 and Canadian Army Mess Tin Rations", made by the Armored Medical Research Laboratory, Fort Knox, Kentucky.

TABLE 5

DISTRIBUTION OF VALUES FOR FASTING URINARY EXCRETION OF

SODIUM CHLORIDE

Category	Number of subjects	Gms NaCl per hour 0.01 to 0.10	0.11 to 0.20	0.21 to 0.30	0.31 to 0.40	0.41 to 0.50	0.51 to 0.60	0.61 to 0.70	0.71 to 0.80	0.81 to 0.90	0.91 to 1.00
		(Per cent of subjects)									
All subjects	111	6.3	18.9	21.6	15.3	17.2	9.9	3.6	3.6	2.7	0.9
High B_1 & B_2 Score	20	5.0	25.0	5.0	35.0	15.0	15.0	---	---	---	---
High B_1 Score	23	8.8	26.0	4.4	21.7	17.3	13.0	4.4	4.4	---	---
High B_2 Score	21	4.7	28.5	9.4	24.4	14.2	9.4	---	4.7	---	4.7
Low B_1 & B_2 Score	20	10.0	25.0	25.0	10.0	15.0	10.0	5.0	---	---	---
Low B_1 Score	20	5.0	20.0	25.0	25.0	15.0	5.0	5.0	---	---	---
Low B_2 Score	20	15.0	15.0	30.0	5.0	10.0	10.0	5.0	10.0	---	---

The average excretion by the entire group --- 0.36 gms NaCl per fasting hour.

TABLE 6

DISTRIBUTION OF VALUES FOR PLASMA PROTEIN CONCENTRATION

Category	Number of subjects	Gms of protein per 100 cc. 5.8 to 6.1	6.2 to 6.4	6.5 to 6.8	6.9 to 7.1	7.2 to 7.4	7.5 to 7.7
		(Per cent of subjects)					
All Subjects	109	0.9	12.8	40.4	28.4	13.8	2.7
High B_1 & B_2 Score	19	---	31.5	36.9	15.8	15.8	---
High B_1 Score	21	4.9	28.5	14.3	28.5	23.8	---
High B_2 Score	20	---	25.0	40.0	20.0	15.0	---
Low B_1 & B_2 Score	20	---	20.0	40.0	25.0	15.0	---
Low B_1 Score	20	---	15.0	40.0	25.0	20.0	---
Low B_2 Score	20	---	20.0	35.0	30.0	15.0	---

The average concentration -- 6.7 gms per 100 cc.

TABLE 7

DISTRIBUTION OF VALUES FOR HEMOGLOBIN CONCENTRATION

Category	Number of Sub'ts	12.2 to 12.9	13.0 to 13.7	13.8 to 14.5	14.6 to 15.3	15.4 to 16.1	16.2 to 16.9	17.0 to 17.7	17.8 to 18.5	18.6 to 19.3	19.4 to 20.1
		Gms of Hemoglobin per 100 cc.									
		(Per cent of subjects)									
All Subjects	107	0.9	----	6.5	15.0	30.0	30.0	7.5	5.5	1.9	1.9
High B_1 & B_2 Score	19	----	----	----	5.3	10.5	52.7	20.9	----	5.3	5.3
High B_1 Score	21	----	----	14.3	4.8	19.0	33.3	14.3	4.8	4.8	4.8
High B_2 Score	20	----	----	----	5.0	10.0	55.0	10.0	10.0	5.0	5.0
Low B_1 & B_2 Score	19	----	----	15.8	5.3	47.3	20.9	----	10.5	----	----
Low B_1 Score	18	----	----	16.6	11.1	33.3	22.2	----	11.1	----	5.7
Low B_2 Score	19	----	----	15.8	15.8	36.8	20.9	5.3	5.3	----	----

The average hemoglobin concentration --
16.0 gms per 100 cc.

TABLE 8

DISTRIBUTION OF VALUES FOR URINARY EXCRETION OF VITAMIN C. (ASCORBIC ACID).

Category	Number of subjects	Mgms. of ascorbic acid per fasting hour. 0.11 to 0.20	0.21 to 0.30	0.31 to 0.40	0.41 to 0.50	0.51 to 0.60	0.61 to 0.70	0.71 to 0.80	0.81 to 0.90	0.91 to 1.00	1.01 to 1.50	0.51 to 2.00
		(Per cent of subjects)										
All Subjects	100	9.0	13.0	17.0	15.0	10.0	11.0	12.0	6.0	2.0	6.0	2.0
High B_1 & B_2 Score	20	5.0	25.0	5.0	15.0	15.0	10.0	10.0	5.0	----	10.0	----
High B_1 Score	22	9.1	9.1	22.9	27.2	9.1	13.6	4.5	----	----	4.5	----
High B_2 Score	21	9.5	19.0	9.5	9.5	14.2	19.0	9.5	4.9	----	4.9	----
Low B_1 & B_2 Score	20	15.0	10.0	20.0	15.0	10.0	5.0	10.0	10.0	5.0	----	----
Low B_1 Score	20	10.0	15.0	15.0	20.0	10.0	5.0	15.0	5.0	----	5.0	----
Low B_2 Score	20	25.0	15.0	25.0	15.0	5.0	----	5.0	10.0	----	----	----

The average excretion of all subjects -- 0.54 mgms per hour.

TABLE 9

DISTRIBUTION OF VALUES FOR WHOLE BLOOD* CONCENTRATIONS OF VITAMIN C. (ASCORBIC ACID)

Category	Number of Subjects	Mgms of ascorbic acid per 100 cc. 0.21 to 0.30	0.31 to 0.40	0.41 to 0.50	0.51 to 0.60	0.61 to 0.70	0.71 to 0.80	0.81 to 0.90	0.91 to 1.00	1.01 to 1.10	1.11 to 1.20	1.21 to 1.30	1.31 to 1.40	1.4 to 1.6
		(Per cent of subjects)												
All Subjects	99	11.0	14.0	17.0	22.0	12.0	9.0	4.0	6.0	2.0	----	1.0	1.0	1.0
High B_1 & B_2 Score	18	22.2	5.6	16.6	16.6	16.6	11.2	----	11.2	----	----	----	----	----
High B_1 Score	19	15.8	10.5	10.5	26.4	10.5	10.5	----	15.8	----	----	----	----	----
High B_2 Score	21	24.1	14.2	19.0	9.5	14.2	9.5	----	9.5	----	----	----	----	----
Low B_1 & B_2 Score	18	5.6	44.4	11.1	16.6	5.6	11.1	----	----	----	----	----	5.6	----
Low B_1 Score	18	16.6	33.3	11.1	11.1	5.6	11.1	----	----	5.6	----	----	5.6	----
Low B_2 Score	18	5.6	27.7	16.6	11.1	22.2	5.6	----	5.6	----	----	----	5.6	----

The average whole blood concentration of ascorbic acid --- 0.61 mgms per 100 cc.

*Instances where only plasma Vitamin C was determined, 0.05 mgms was added to give the whole blood concentration in the above table.

TABLE 10

DISTRIBUTION OF VALUES FOR FASTING URINARY EXCRETION OF THIAMINE (B_1)

Micrograms excreted per fasting hour	Per cent of subjects
0.0 to 1.0	1.8
1.1 to 2.0	3.6
2.1 to 3.0	10.8
3.1 to 4.0	17.2
4.1 to 5.0	19.8
5.1 to 6.0	13.5
6.1 to 7.0	9.9
7.1 to 8.0	6.3
8.1 to 9.0	5.4
9.1 to 10.0	2.7
10.1 to 11.0	2.7
11.1 to 12.0	2.7
12.1 to 13.0	0.9
13.1 to 14.0	0.9
14.1 to 15.0	---
15.1 to 16.0	0.9
16.1 and up	0.9

The average of 110 subjects -- 5.65 mcgm per fasting hour.

TABLE 11

DISTRIBUTION OF THIAMINE EXCRETION VALUES AFTER A TEST DOSE OF 5000 MICROGRAMS.

Micrograms excreted per 4 hours	Per cent of subjects
0 to 50	9.4
51 to 100	27.3
101 to 150	21.7
151 to 200	15.1
201 to 250	10.4
251 to 300	6.7
301 to 350	1.9
351 to 400	0.9
401 to 450	1.9
451 to 500	1.9
501 to 550	1.9
551 and up	0.9

The average excretion -- 160 micrograms per 4 hours.

TABLE 12

DISTRIBUTION OF VALUES FOR FASTING URINARY EXCRETION OF

RIBOFLAVIN (B_2)

Micrograms excreted per fasting hour	Per cent of subjects
0.0 to 2.0	9.9
2.1 to 4.0	6.9
4.1 to 6.0	12.9
6.1 to 8.0	15.9
8.1 to 10.0	16.9
10.1 to 12.0	6.9
12.1 to 14.0	6.9
14.1 to 16.0	5.9
16.1 to 18.0	5.9
18.1 to 20.0	---
20.1 to 30.0	6.9
30.1 to 40.0	2.0
40.1 to 50.0	2.0
50.1 to 60.0	---
60.1 to 70.0	---
70.1 to 80.0	---
80.1 an up	1.0

The average excretion for 101 subjects -- 11.2 micrograms per fasting hour.

TABLE 13

DISTRIBUTION OF RIBOFLAVIN EXCRETION VALUES AFTER A TEST

DOSE OF 5000 MICROGRAMS.

Micrograms excreted per 4 hours	Per cent of subjects
0 to 200	6.1
201 to 400	12.3
401 to 600	14.2
601 to 800	13.3
801 to 1000	21.5
1001 to 1200	11.2
1201 to 1400	13.3
1401 to 1600	2.0
1601 to 1800	4.1
1801 to 2000	1.0
2001 an up	1.0

The average excretion for 98 subjects, 829 micrograms per 4 hours.

TABLE 14

LOAD TEST PERFORMANCE LIMITS IN HIGH AND LOW SCORE GROUPS

Category	Number of subjects	Vitamin B_1 Limit* (micrograms)		Vitamin B_2 Limit** (micrograms)	
High B_1 & B_2 Score	20	Above	150	Above	1070
High B_1 Score	23	Above	200	No limit	
High B_2 Score	21	No limit		Above	1200
Low B_1 & B_2 Score	20	Below	85	Below	510
Low B_1 Score	20	Below	65	No limit	
Low B_2 Score	20	No limit		Below	415
Level of performance suggestive of severe deficiency (Johnson, et al)		Below	50	Below	200

*Based on 4 hour excretion of thiamine after a 5000 microgram test dose of crystaline vitamin.

**Based on 4 hour excretion of riboflavin after a 5000 microgram test dose of crystaline vitamin.

TABLE 15

AVERAGE PERFORMANCE* IN LOAD TESTS BY HIGH AND LOW

SCORE GROUPS.

Category	Number of subjects	Vitamin B_1 average Performance (micrograms)	Vitamin B_2 average performance (micrograms)
All Subjects	98	160	829
High B_1 & B_2 Score	20	213	1376
High B_1 Score	23	278	1016
High B_2 Score	21	180	1466
Low B_1 & B_2 Score	20	85	314
Low B_1 Score	20	46	456
Low B_2 Score	20	89	266
Score suggestive of severe dificiency (Johnson, et. al.)		Below 50	Below 200

*Performance refers to the micrograms of the vitamin recovered in a 4-hour urine excretion immediately following a 5000 microgram test dose.

TABLE 16

DISTRIBUTION OF SUBJECTIVE RATINGS ON MORALE.

Category	Number of Sub'ts	Ratings* Excellent	Good	Fair	Poor
		(Per cent of subjects)			
All Subjects	111	3.6	34.3	41.4	20.7
High B_1 & B_2 Score	20	10.0	65.0	25.0	----
High B_1 Score	23	8.7	43.5	43.5	4.3
High B_2 Score	21	9.6	52.3	33.3	4.8
Low B_1 & B_2 Score	20	----	40.0	40.0	20.0
Low B_1 Score	20	5.0	35.0	40.0	20.0
Low B_2 Score	20	----	20.0	50.0	30.0

*Ratings were made by the individual subject in a confidential questionaire.

TABLE 17

DISTRIBUTION SUBJECTIVE RATINGS ON APPETITE

Category	Number of Sub'ts	Excellent	Ratings* Good	Fair	Poor
		(Per cent of subjects)			
All Subjects	111	4.5	39.6	38.8	17.1
High B_1 & B_2 Score	20	---	65.0	25.0	10.0
High B_1 Score	23	8.7	39.2	34.8	17.4
High B_2 Score	21	4.8	57.1	28.6	9.5
Low B_1 & B_2 Score	20	----	35.0	45.0	20.0
Low B_1 Score	20	----	35.0	35.0	30.0
Low B_2 Score	20	5.0	25.0	45.0	25.0

*Ratings were made by the individual subject in a confidential questionaire.

TABLE 18

INCIDENCE AND DISTRIBUTION OF DISORDERS COMPILED FROM
THE CONFIDENTIAL QUESTIONAIRE

Category	Number of Sub'ts	Having no Disorder	Having one or more Disorders*	Distribution of disorders				
				Skin	Acute Intest.	Dengue Fever	Malaria Fever	Hepe-Eitis
			(Per cent of group)					
All Subjects	101	76.5	23.5	17.0	15.0	3.0	3.0	1.0
High B_1 & B_2 Score	20	80.0	20.0	15.0	10.0	----	---	---
High B_1 Score	23	82.7	17.3	21.7	4.3	4.3	---	---
High B_2 Score	21	85.7	14.3	9.5	4.9	----	---	---
Low B_1 & B_2 Score	20	60.0	40.0	30.0	30.0	10.0	10.0	---
Low B_1 Score	20	60.0	40.0	25.0	25.0	5.0	5.0	---
Low B_2 Score	20	55.0	45.0	20.0	40.0	10.0	5.0	---

*Some of the subjects had multiple disorders.

TABLE 19

MEDICAL HISTORY* OF HIGH SCORE GROUP FOR PREVIOUS SIX (6) MONTHS

Subject Number	Hospital-izations	Sick Calls	Acute Resp. Infect.	Acute Diar-rheas	Insom-nia	Head-aches	Endur-ance	Faintness on Arising
II-2	0	1	0	1	0	0	0	0
II-3	0	0	0	1	0	0	0	0
III-2	0	3	2	0	0	0	0	0
IV-2	0	3	3	0	0	0	0	0
IV-3	0	4	0	0	0	0	0	0
IV-4	0	0	0	0	0	0	0	0
VI-10	0	1	0	0	0	0	0	0
VII-7	0	6	0	0	0	0	0	0
VII-8	0	3	5	0	0	0	0	0
IX-2	0	3	0	0	0	0	L	0
IX-9	0	0	0	2	0	0	0	0
X-3	1	4	1	0	0	0	0	0
X-4	0	0	2	0	0	0	0	0
X-5	0	0	0	0	0	0	0	0
X-10	0	0	0	0	0	0	0	0
XI-5	0	0	1	0	0	0	L	0
Total	1	28	9	4	0	0	2	0
Number per man	0.06	1.75	0.56	0.25				
Per cent Incidence	6.2	56.2	37.5	18.7			12.5	

0 -- None or normal
+ -- Positive or present
L -- Below normal

*Taken by clinicians during physical examination.

TABLE 20

MEDICAL HISTORY* OF LOW SCORE GROUP FOR PREVIOUS SIX (6) MONTHS

Subject Number	Hospital-izations	Sick Calls	Acute Resp. Infect.	Acute Diar-rheas	Insom-nia	Head-aches	Endur-ance	Faintness on Arising
II-7	0	8	0	0	0	0	0	0
III-10	0	0	1	0	0	0	0	0
IV-8	1	1	5	1	0	0	0	0
IV-9	0	7	10	0	0	0	L	0
V-3	0	0	0	1	0	0	0	0
V-5	0	2	1	6	0	0	0	0
V-6	0	1	4	0	0	+	L	0
V-8	1	3	1	7	0	0	0	0
VI-1	0	6	1	0	0	0	0	0
VI-11	0	1	3	0	0	0	0	0
VII-10	0	1	4	4	0	+	0	0
VII-11	2	15	2	2	+	+	L	0
VIII-2	0	6	6	6	0	0	0	0
VIII-3	0	2	2	1	0	+	L	+
VIII-5	1	4	1	3	0	0	0	0
VIII-6	0	4	0	1	0	0	0	0
VIII-7	0	3	3	1	0	+	0	0
VIII-9	0	0	0	6	0	0	0	0
IX-1	0	0	1	0	0	0	0	0
IX-3	0	3	1	2	0	0	L	+

TABLE 20 (Cont.)

MEDICAL HISTORY* OF LOW SCORE GROUP FOR PREVIOUS SIX (6) MONTHS

Subject Number	Hospital-izations	Sick Calls	Acute Resp. Infect.	Acute Diar-rheas	Insom-nia	Head-aches	Endur-ance	Faintness on Arising
IX-5	1	0	1	0	+	0	L	0
IX-7	1	2	0	1	+	0	L	+
IX-8	0	2	1	1	0	0	0	0
IX-10	1	8	6	10	0	0	L	+
X-2	0	15	C	1	+	0	0	+
X-6	0	1	1	1	0	0	L	0
XI-2	1	12	C	0	0	0	0	0
Total	9	107	54	57	4	5	9	5
Number per man	0.33	3.96	2.0	2.11				
Per cent Incidence	30.0	81.5	77.7	74.1	14.8	18.5	33.3	18.5

0 -- None or normal
+ -- Positive or present
C -- Chronic
L -- Below normal

*Taken by clinicians during physical examination.

TABLE 21

CLINICAL NERVOUS AND OCULAR SYMPTOMS OF NUTRITIONAL DEFICIENCY FOR THE HIGH SCORE GROUP.

Subject Number	Paraes-thesia	Nerve Tender-ness	Leg Tendon Reflexes	Eye-History	Conjunct-ivitis	Vascular-ization of Cornea
II-2	0	0	+	0	0	0
II-3	0	0	+	0	0	0
III-2	0	0	+	0	0	0
IV-2	0	0	+	0	+	0
IV-3	0	0	+	0	0	0
IV-4	0	0	+	0	0	+
VI-10	0	0	+	0	0	0
VII-7	0	0	+	0	+	0
VII-8	0	0	+	L	0	0
IX-2	0	0	+	0	+	+
IX-9	0	0	+	0	0	0
X-3	0	0	+	0	0	0
X-4	0	0	+	0	+	0
X-5	0	0	+	0	+	0
X-10	0	0	+	0	0	0
XI-5	0	0	+	0	+	0
Total	2	0	16	1	5	2
Per cent incidence	12.5	.0	100	6.2	31.2	12.5

0 -- None or normal
+ -- Positive or present
L -- Lacrimation

TABLE 22

CLINICAL NERVOUS AND OCULAR SYMPTOMS OF NUTRITIONAL DEFICIENCY FOR THE LOW SCORE GROUP

Subject Number	Paraes-thesia	Nerve Tender-ness	Leg Tendon Reflexes	Eye-History	Conjunct-ivitis	Vascular-ization of Cornea
II-7	0	0	+	0	+	0
III-10	0	0	+	0	+	0
IV-8	0	0	+	0	+	0
IV-9	+	0	0(P)	0	0	0
V-3	0	0	+	0	0	0
V-5	0	0	+	B,PH	+	0
V-6	0	0	+	0	+	0
V-8	0	0	0	0	0	0
VI-1	0	0	+	0	0	0
VI-11	0	0	+	0	0	0
VII-10	0	0	+	0	0	+
VII-11	0	0	+	0	+	0
VIII-2	0	0	+	0	+	+
VIII-3	0	0	+	B,L	+	0
VIII-5	0	0	+	0	0	0
VIII-6	0	0	+	0	0	0
VIII-7	0	0	+	0	+	0
VIII-9	0	0	+	0	0	0
IX-1	0	0	+	0	+	0
IX-3	0	0	+	0	0	0

TABLE 22 (Cont.)

CLINICAL NERVOUS AND OCULAR SYMPTOMS OF NUTRITIONAL DEFICIENCY FOR THE LOW SCORE GROUP

Subject Number	Paraes- thesia	Nerve Tender- ness	Leg Tendon Reflexes	Eye- History	Conjunct- ivitis	Vascular- ization of Cornea
IX-5	0	0	+	PH	0	±
IX-7	0	+	+	0	0	0
IX-8	0	0	+	0	+	0
IX-10	0	±	+	0	0	0
X-2	0	0	+	0	0	+
X-6	0	0	+	0	0	0
XI-2	0	0	0*	0	0	0
Total	6	2	3	3	11	4
Per cent incidence	22.2	7.4	11.1	11.1	40.7	14.8

0 -- None of normal
+ -- Positive or present
± -- Undecided
P -- Patellar reflexes
PH - Photophobia
L -- Lacrimation
B -- Burning

*One side only.

TABLE 23

CLINICAL NUTRITIONAL DEFICIENCY SYMPTOMS OF THE MOUTH AND ALSO LIVER ENLARGEMENT FOR THE HIGH SCORE GROUP

Subject Number	Bleeding Gums	Gingivitis	Glossitis	Sore Tongue	Mouth Sores	Cheilosis	Liver Enlargement
II-2	0	0	0	0	0	0	0
II-3	0	0	0	0	0	±	0
III-2	0	0	0	0	0	0	0
IV-2	0	0	0	0	0	±	0
IV-3	0	+	0	0	0	0	0
VI-4	0	0	+	0	0	0	0
VI-10	+	0	+	0	0	0	0
VII-7	0	0	0	0	0	0	0
VII-8	+	0	0	0	0	0	0
IX-2	0	0	0	0	0	0	1F
IX-9	0	0	+	0	+	0	0
X-3	0	0	+	0	0	0	0
X-4	0	0	0	0	0	0	0
X-5	0	0	0	0	0	0	0
X-10	0	0	0	0	0	0	0
XI-5	0	0	0	0	0	0	0
Total	2	1	4	0	1	2	1
Per cent incidence	12.5	6.2	25.0	0	6.2	12.5	6.2

0 -- None or normal
+ -- Positive or present
± -- Undecided
F -- Finger-widths enlargement.

TABLE 24

CLINICAL NUTRITIONAL DEFICIENCY SYMPTOMS OF THE MOUTH
AND ALSO LIVER ENLARGEMENT FOR THE LOW SCORE GROUP

Subject Number	Bleeding Gums	Gingivitis	Glossitis	Sore Tongue	Mouth Sores	Cheilosis	Liver Enlargement
II-7	0	0	0	0	0	0	0
III-10	0	0	0	0	0	0	0
IV-8	0	0	0	0	0	+	0
IV-9	+	0	0	0	0	0	0
V-3	0	0	0	0	0	0	0
V-5	0	0	+	0	0	0	0
V-6	0	0	0	+	0	0	1F
V-8	0	0	0	0	0	0	0
VI-1	0	±	+	0	0	0	1F
VI-11	0	0	+	0	+	0	0
VII-10	0	0	±	0	0	0	0
VII-11	+	0	0	0	+	±	T
VIII-3	0	0	0	0	0	0	0
VIII-2	0	0	+	0	0	0	0
VIII-5	+	+	0	0	0	0	0
VIII-6	+	0	0	0	0	±	0
VIII-7	0	0	0	0	0	0	0
VIII-9	0	+	0	0	0	0	0
IX-1	0	0	+	0	0	0	0
IX-3	+	0	+	0	0	±	1F

TABLE 24 (Cont.)

CLINICAL NUTRITIONAL DEFICIENCY SYMPTOMS OF THE MOUTH AND ALSO LIVER ENLARGEMENT FOR THE LOW SCORE GROUP

Subject Number	Bleed-ing	Ging-ivitis	Glos-sitis	Sore Tongue	Mouth Sores	Cheilosis	Liver Enlarge-ment
IX-5	+	0	+	0	0	0	0
IX-7	0	0	±	0	0	±	1F
IX-8	0	0	0	0	0	0	0
IX-10	0	0	+	0	+	±	1½F
X-2	0	0	0	0	0	0	2F
X-6	0	0	[illegible]	0	0	0	0
XI-2	0	0	0	0	0	0	0
Total	5	3	10	1	3	6	6
Per cent incidence	18.5	11.1	37.0	3.7	11.1	22.2	22.2

0 -- None or normal
+ -- Positive or present
± -- Undecided
T -- Liver tender but not palpable
F -- Finger-widths enlargement

TABLE 25

INCIDENCE AND DISTRIBUTION OF CLINICALLY DIAGNOSED
SKIN DISORDERS FOR HIGH SCORE GROUP.

Subject Number	Acne	Impetigo	Non-infectious Dermatitis					
			Pellagraous	Riboflavin (facial)	Pubic	Scrotal	Perianal*	Seborrheic
II-2	0	0	0	0	+	0	0	0
II-3	0	0	0	0	0	0	0	+
III-2	+	0	0	0	0	0	0	+
IV-2	0	0	0	0	0	+	0	0
IV-3	0	0	0	0	R	0	0	0
VI-4	0	0	0	0	R	0	R	0
VI-10	0	0	0	0	0	+	0	0
VII-7	0	+	0	0	R	0	+	0
VII-8	0	0	0	0	0	+	0	0
IX-2	0	0	0	0	R	0	+	0
IX-9	0	0	0	0	+	+	+	+
X-3	0	0	0	0	0	0	0	F
X-4	0	0	0	0	R	0	0	0
X-5	0	0	0	0	R	0	0	0
X-10	0	0	0	0	0	0	0	0
XI-5	0	0	0	0	0	-	0	0
Total	1	1	0	0	8	5	4	4
Per cent Incidence	6.2	6.2			50.0	31.2	14.8	14.8

0 -- None or normal
+ -- Positive or present
R -- Ringworm
F--- Folliculitis

*Lession found in sacro-coccygeal region between buttocks.

TABLE 26

INCIDENCE AND DISTRIBUTION OF CLINICALLY DIAGNOSED
SKIN DISORDERS FOR LOW SCORE GROUP

Subject Number	Acne	Impetigo	Non-infectious Dermatitis					
			Pellagraous	Riboflavin (facial)	Pubic	Scrotal	Perianal*	Seborrheic
II-7	0	++	0	0	0	0	0	F
III-10	0	0	0	0	0	+	0	F
IV-8	0	0	0	0	0	+	[illegible]	0
IV-9	0	0	0	0	0	+	+	0
V-3	0	0	0	0	+	0	+	0
V-5	0	0	0	0	0	++	+	0
V-6	0	0	0	0	R	0	+	0
V-8	0	0	0	0	R	+	+	0
VI-1	0	0	0	±	0	+	+	0
VI-11	0	0	0	0	0	0	+	F
VII-10	0	0	0	0	0	0	0	0
VII-11	0	0	0	0	0	0	0	0
VIII-2	++	0	0	0	0	+	+	+
VIII-3	0	0	0	0	0	0	0	+
VIII-5	0	0	0	0	0	0	0	0
VIII-6	0	0	0	0	0	0	0	0
VIII-7	0	0	0	0	0	+	+	0
VIII-9	++	0	0	0	0	+	0	F
IX-1	0	0	0	0	0	0	+	0
IX-3	0	0	0	0	0	0	0	0

TABLE 26 (Cont.)

INCIDENCE AND DISTRIBUTION OF CLINICALLY DIAGNOSED
SKIN DISORDERS FOR LOW SCORE GROUP

Subject Number	Acne	Impetigo	Non-infectious Dermatitis					
			Pellagraous	Riboflavin (facial)	Pubic	Scrotal	Perianal*	Seborrheic
IX-5	+	0	0	0	0	0	0	0
IX-7	0	0	0	0	0	0	0	0
IX-8	0	0	0	0	R	0	0	0
IX-10	0	0	0	0	0	+	+	+
X-2	0	0	0	0	0	+	+	0
X-6	0	0	0	0	0	0	0	0
XI-2	0	+	0	0	0	0	0	0
Total	2	2	0	1	4	11	12	7
Per cent Incidence	7.4	7.4		3.7	14.8	40.3	44.4	26.0

0 — None or normal
+ -- Positive or present
± -- Undecided
R -- Ringworm
F -- Folliculitis

*Lesion found in sacro-coccygeal area between buttocks.

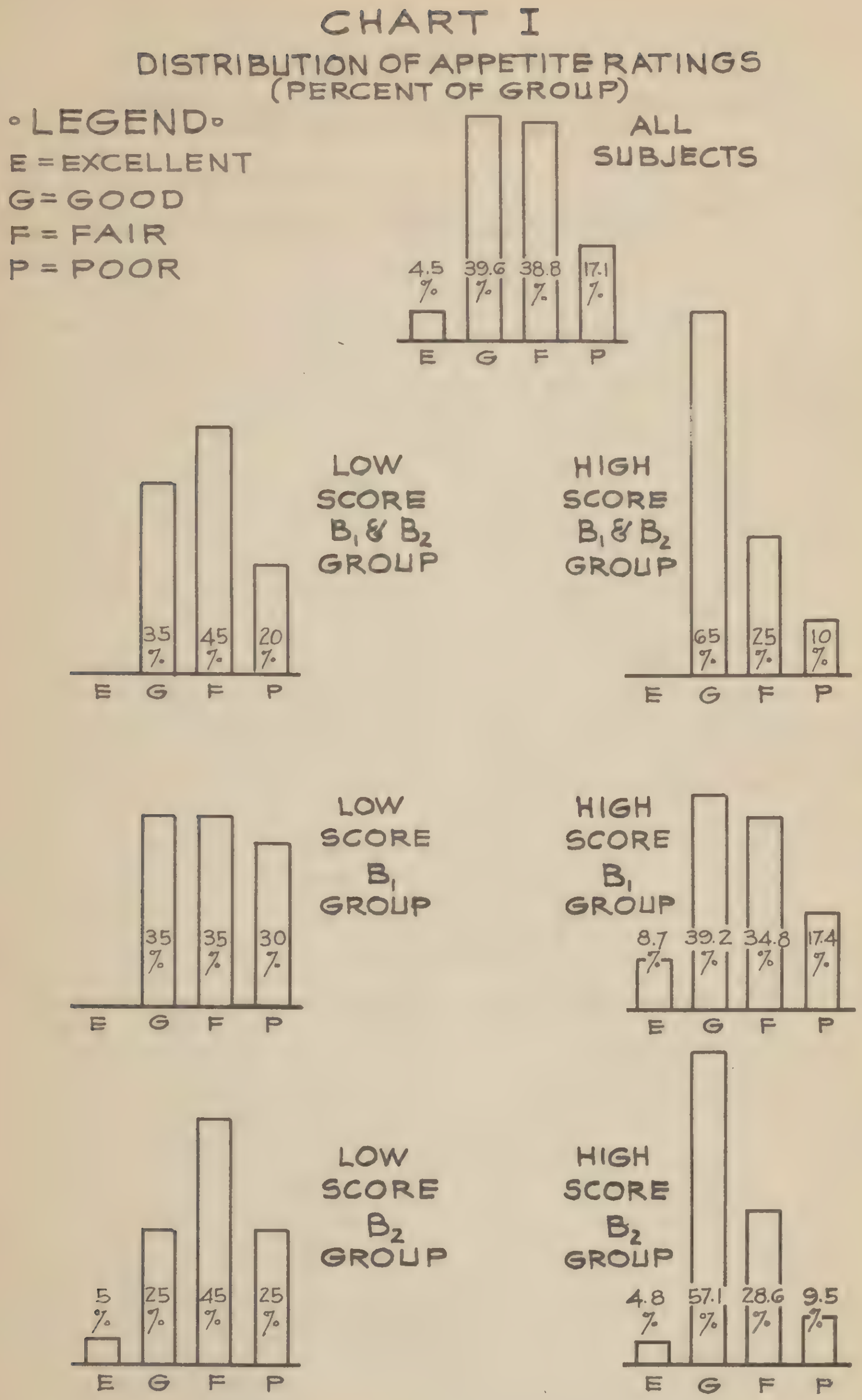
CHART I
DISTRIBUTION OF APPETITE RATINGS
(PERCENT OF GROUP)
LEGEND
E = EXCELLENT
G = GOOD
F = FAIR
P = POOR
ALL SUBJECTS
4.5 %
39.6 %
38.8 %
17.1 %
E G F P
LOW SCORE B1 & B2 GROUP
35 %
45 %
20 %
E G F P
HIGH SCORE B1 & B2 GROUP
65 %
25 %
10 %
E G F P
LOW SCORE B1 GROUP
35 %
35 %
30 %
E G F P
HIGH SCORE B1 GROUP
8.7 %
39.2 %
34.8 %
17.4 %
E G F P
LOW SCORE B2 GROUP
5 %
25 %
45 %
25 %
E G F P
HIGH SCORE B2 GROUP
4.8 %
57.1 %
28.6 %
9.5 %
E G F P

CHART II
DISTRIBUTION OF MORALE RATING
(PERCENT OF GROUP)

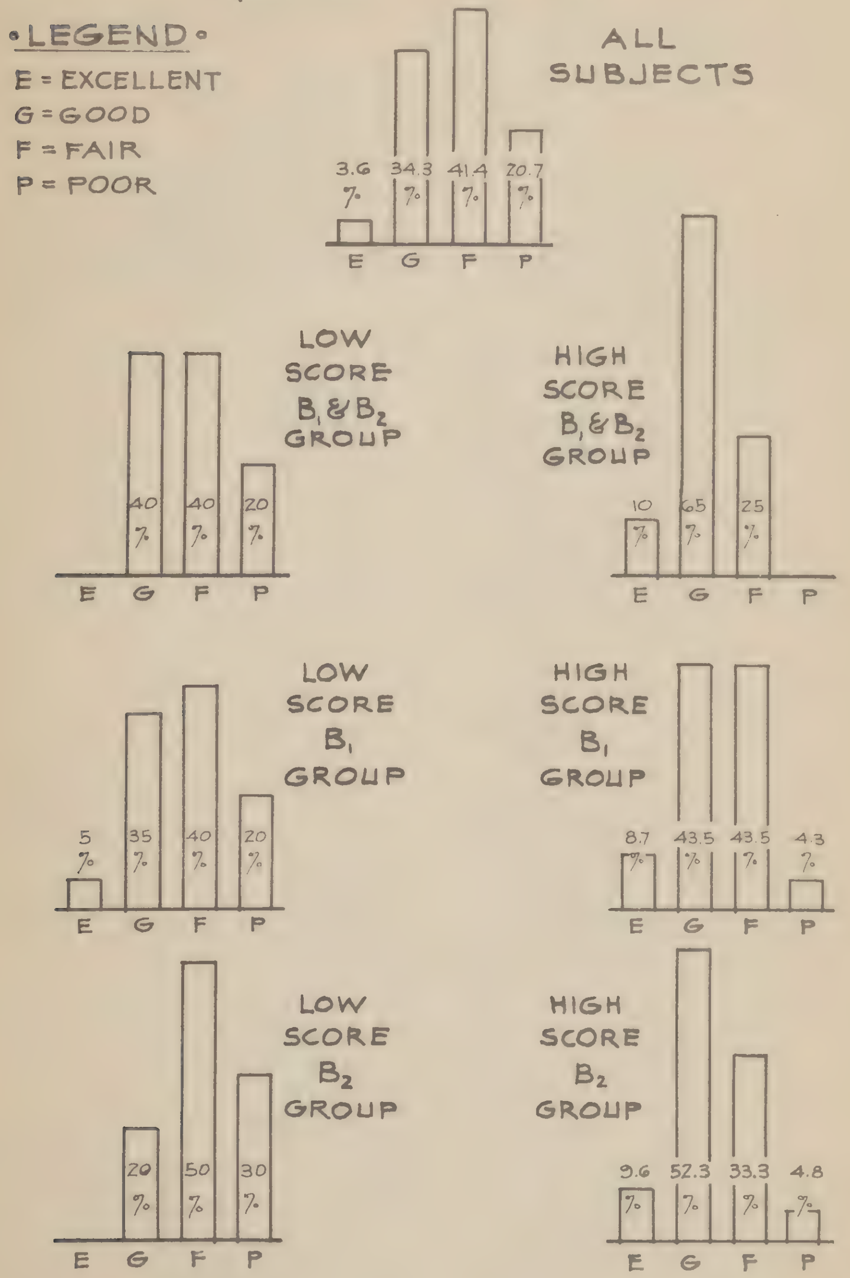

CHART III

DISTRIBUTION OF HEMOGLOBIN VALUES
EXPRESSED IN
(GRAMS PER 100 CC AND PERCENT OF GROUP)

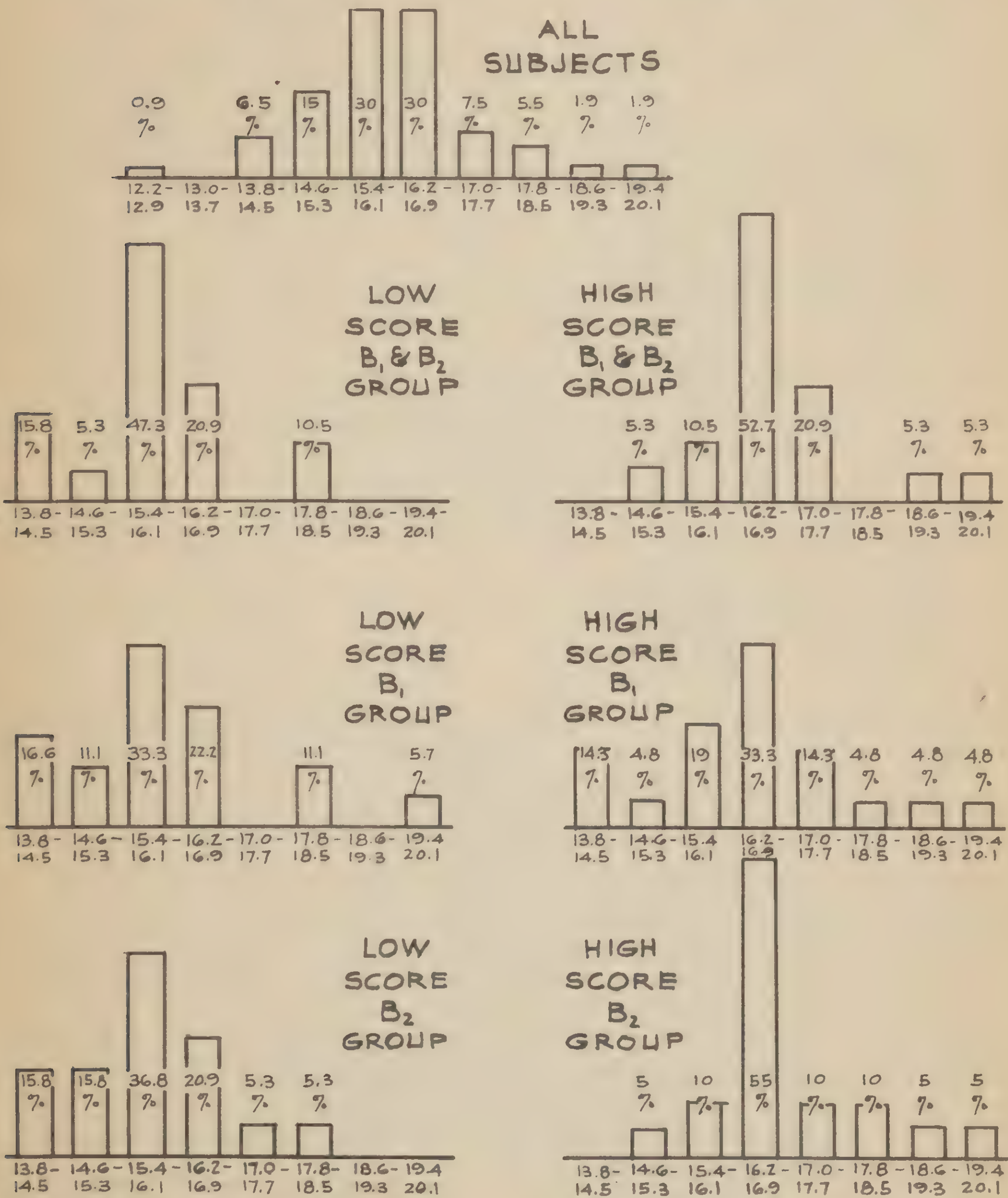

CHART IV

DISTRIBUTION OF B-VITAMIN URINARY EXCRETIONS

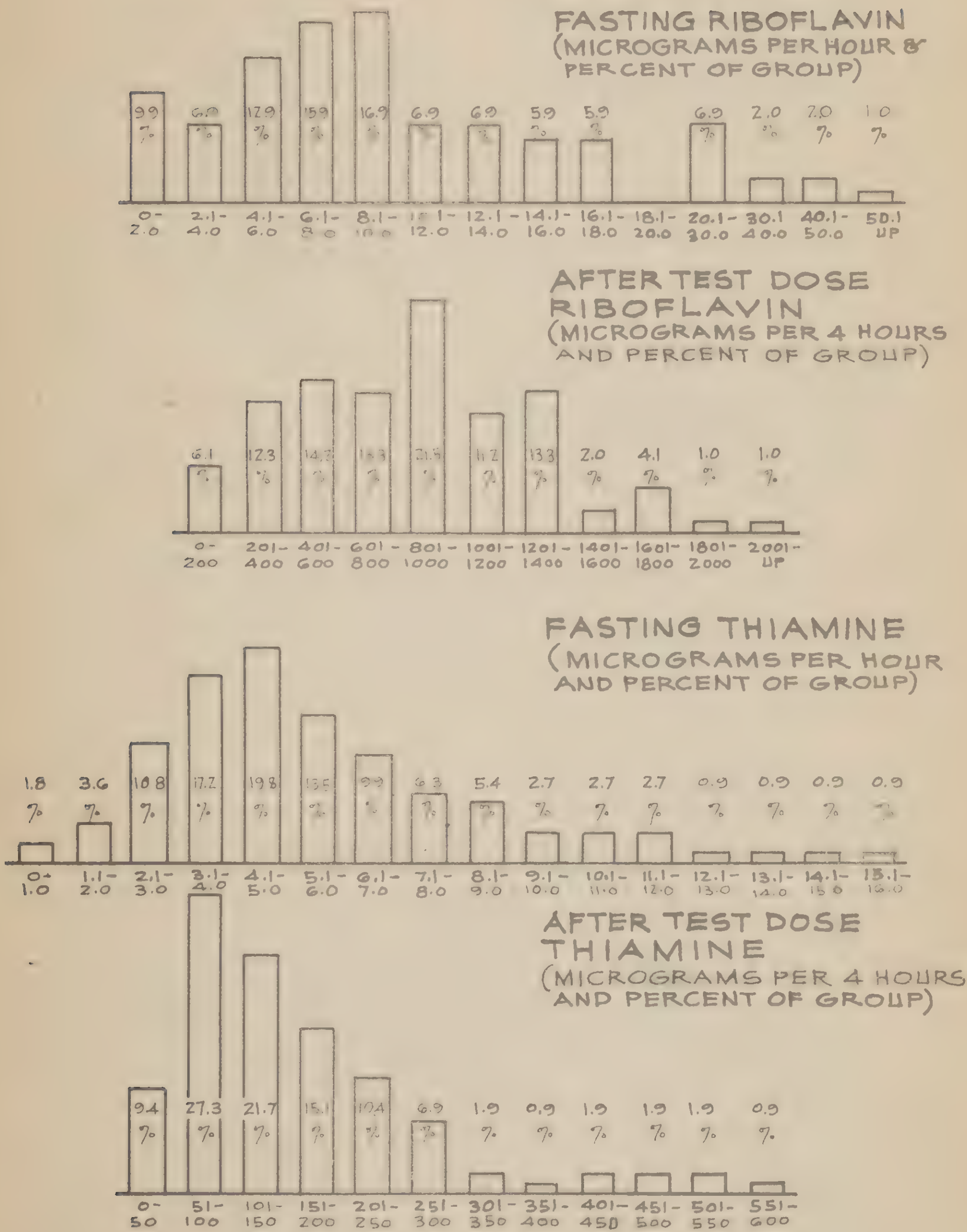

CHART V
DISTRIBUTION OF VALUES